

nature

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Catch 22

WE don't often comment editorially on the stories of Soviet policy towards dissidents that we carry with monotonous regularity. This is not because we don't believe them—it's because they have a peculiar eloquence of their own to which cosy editorialising from a London office can add little. Sufficient it is that scientists should be aware on a worldwide basis of what goes on and act accordingly. The same goes for the testimony of Vladimir Bukovskii on the following page. But one story emerging from Moscow so confounded our sense of logic that we feel it deserves a little comment.

Academician Veniamin Levich was briefly taken into custody on December 21 as part of a new campaign against participation in the unofficial scientific seminars for dismissed scientists. He was referred to a TASS statement of November 9 concerning the Soviet position on emigration. Since this provoked no overseas reactions, he was told, this must imply tacit agreement.

The unattributed statement, one of many issued on or around Revolution Day, was broadcast in English at 07.36 and ran, in part, "each request from an individual citizen to be allowed to go and live in another country in order to reunite split families is given full consideration. Refusals

are made in an insignificant number of cases, when the citizen expressing the wish to leave the USSR has had access in the course of his duties to works or information that represent a state secret . . ."

Since "the secrets of a state are always its own exclusive property", it went on, "it is an internal question for the Soviet Union to decide which specific works or information are to be considered secret, obsolete, or to have lost importance in guaranteeing the defences and other important national interests of the USSR". Accordingly, "any attempt at pressuring competent Soviet organisations into changing their position is considered an interference in the domestic affairs of the USSR. As for the big anti-Soviet noise raised in the West, it pursues the sole aim of misleading public opinion . . . Bourgeois propaganda is doing everything it can to distract attention from the systematic and gross violations of basic human rights in capitalist society".

In view of the strong hints that a big anti-Soviet noise is an interference in the domestic affairs of the USSR it is somewhat remarkable that the said "competent Soviet authorities" now quote the lack of "noise raised in the West" as a justification for their policy. □

No export potential in steam tape-recorders

"PARLEZ-VOUS electric?", runs a recent large advertisement by the UK Electricity Council in national newspapers, at a cost of goodness-knows-how-many-thousand pounds. "There are thousands of tape-recorders with headsets in use in UK schools. Thousands more are used each year by executives of multi-national companies on six-week intensive courses in a foreign language. Headsets. Video tape-recording equipment. Magnetic tape. All these modern educational aids are powered by electricity. The child learns his language idiomatically. So do the men. Men responsible for earning this country millions of pounds in export orders from foreign countries. Exports are this country's life blood. Electricity helps generate them. **Think Electric**".

"Look here, Blenkinsop," said the Minister for Energy, the moment the Director of the Electric Power Generating Board had sat down. Blenkinsop knew immediately that this was to be the dreaded eyeball-to-eyeball confrontation. He fumbled for his pipe. "I'm getting fed up with the way everyone else in the Cabinet is talking about export, export all the time—boasting about their departments' achievements—and we're still importing in our department. I get a lot of stick for it, so you'd better get your advertising copywriters to do something about brushing up our image. There must be something we export. Oh, and also", Blenkinsop shifted uneasily, "I've been looking at electric consumption figures for these last few weeks. What's going on? We're 5% down on last year, and I've got all these boiler-makers, miners, nuclear engineers and what-have-you on my back about building more power stations. I want you

to get out and stimulate consumption in a really big way. Find something which really soaks up electricity and push it hard." "Yes, sir" said Blenkinsop. The minister pulled a drawer open. Blenkinsop wondered if it was to be a letter asking for his resignation. But no; the minister decanted three or four small boxes on the table.

"Know what these are?" "No, sir". "Really, Blenkinsop, you're not very alert to the opposition. They are tape recorders—but they don't run on electricity. This is a clockwork one—it punches a tiny paper tape. It's made in Switzerland by all those watchmakers put out of business by digital watches. It sounds a bit like a 78-record, I admit, but you see how they'll develop it. This one's made in France. It runs on lighter-fuel, and I hardly need remind you that North Sea oil doesn't yield lighter-fuel so it'll have to be imported. And this one is made by our own dear old Gas Board; that's a shot across your bows, isn't it, Blenkinsop? Now you get people cracking; they've three big campaigns to do on export, consumption and competition".

"Yes, sir", said Blenkinsop, as his civil-servant brain swung into action "but, sir, if I can save us money with some sort of integrated campaign, do you think I might, er . . . er . . . be so bold as to have a Permanent Secretary's 10' 9" × 7' 3" thick-pile carpet for my office? I only really qualify for the Deputy Secretary's 8' 3" × 6' 4" rug".

"Oh, very well" said the minister, irritably, and out trooped a gleeful Blenkinsop. "Odd people, these civil servants," mused the minister, "I wonder what he meant by an integrated approach . . . I hope he doesn't make a fool of us". □



The Bukovskii perspective

Vladimir Bukovskii, the dissident Soviet biologist released after years in and out of prison and psychiatric hospitals, answers questions from Vera Rich

VR: Do you think that your work in science, studying biology, in any way led you to the protest movement?

VB: I began to take part in the protest movement before I became a student. But my interest in the biological sciences and my studies helped. Science in the USSR (I don't mean the 'social sciences') has followed a path from being completely ideological to being partially so. It is said that at one time mathematicians were forced to prove the class nature of the integral. I did not belong to that period. In my time the physical sciences were already treated with great honour because of their defence potential.

Biology was far less lucky in this respect. Even in 1960, when I entered university, the father of Soviet biology was still Lysenko; genetics and biocybernetics were only just beginning to defend their right to exist. The very methodology of higher education; the abundance of unnecessary Party discipline; the barrack-like spirit; the dependence and the lack of rights of the students, who were blackmailed into joining the Komsomol and informing on their comrades by the threat of losing their grant and expulsion from the university . . . When I, at last, was expelled for "failure to conform with the character of a Soviet student" (for taking part in youth meetings on Mayakovskii Square), I was simply glad, to tell the truth. But my interest in biology remained, and later, even in prison, I continued my self-education as far as possible.

Under the influence of biology and while I was still at school my first disagreement with the ruling doctrine arose. The cornerstone of Marxism, the slogan "Being determines consciousness", evoked active protest in me. Society appeared to me as a living organism, and social consciousness, being the sum of individual consciousness, was the determinant of social processes. This incompatibility gave a stimulus to the analysis, criticism, and rejection of Marxism. Later the very fact of working with science, which develops in a person a careful concern with the truth, a discipline in thought and a training in patience, completed this process. It is not by chance that later there was wide participation of mathematicians, physicists and biologists in our movement. The scientific approach to legal questions, which was established in the first place by mathematician Aleksand Vol'pin, exerted an

enormous influence on the whole development of our movement.

VR: How do you see your own case in the context of the aims of the Sakharov Human Rights Committee?

VB: I consider that the activity of the Sakharov Human Rights Committee in Moscow is a continuation of the process of scientific consideration of the problems of rights and injustices in the Soviet Union about which I have just been speaking. This process, in my opinion, belongs to the man of the future if he, like Andrei Dmitrievich Sakharov, will conscientiously, magnanimously and readily unite to defend all those who are being prosecuted. Like all my comrades in prison, I have always known that Sakharov remembers us, is concerned for us, and defends all of us. In the Vladimir prison we came to know that Sakharov had enumerated dozens of names of political prisoners in his Nobel lecture. For me personally it is clear that the activity of Sakharov and his Human Rights Committee was one of the origins of the wide international campaign for my release.

VR: Taking into account the existence of psychiatric persecution, what do you think is the proper position for Western psychiatrists? Should they boycott all contacts with the Soviet Union? Should they continue to take part in conferences in the Soviet Union, in the hope of exerting unofficial pressure on Soviet psychiatrists? Should the Soviet psychiatric profession be excluded from participation in international conferences until there is a change of policy towards dissidents?

VB: I consider the generalisation "the Soviet psychiatric profession" is not justified in all respects. There are different schools, currents and types of psychiatrists. It is almost impossible to speak of some single policy adopted by all psychiatrists towards dissidents.

In the last examination at the Serbskii Institute in autumn 1971, when, under public pressure, the authorities included among the experts several honest scientists such as Professor Melekhov and Professor Lukomskii, I had no questions regarding the profession's policy towards 'dissidents'. I did not doubt for a second that their decision would be objective.

In 1965 when, on the orders of the KGB, I was taken by force to City Psychiatric Hospital No. 13 in Moscow, young and completely unknown psychiatrists did not fear to declare me to be

sane. Even in psychiatric prisons I met a few decent psychiatrists. Decency in these conditions amounts to heroism. Are you asking me whether I want to exclude these people from the world psychiatric profession, to break off their scientific contacts with you, or yours with them?

On the other hand, is it right to sit down at the same table with Lunts and Snezhevskii, hoping to exert 'unofficial pressure' on them? Their mere presence at the same table as Western psychiatrists gives them respectability, introduces them into the ranks of psychiatrists and makes you accessories to their crimes. By no pressure can you return to them their long-lost consciences. The very idea of behind-the-scenes deals with murderers and executioners is incompatible with the conscience of a true scientist. The 'diplomatisation' of psychiatry has already led to a number of cowardly compromises: contacts between western psychiatrists and odious figures of Soviet psychiatry, and the refusal to discuss openly and to condemn Soviet psychiatric malpractices. It must be understood that the Soviet authorities consider such behaviour to be proof of the West's weakness which only strengthens the repression. It is also very convenient for those in the West who are prepared to have contacts with the Soviet Union at any price.

Is it not remarkable, then, that people educated in the traditions of Western democracy, and accustomed to expressing their opinions vigorously and to considering honour in their relationships with each other, should think it is possible and necessary to conspire, scheme and even compromise their consciences, provided contacts with the Soviet Union are involved? Yet it is not only tactically more correct, humanly speaking it is easier to maintain the direct honourable position of a scientist. One does not have to force one's conscience. No one ever has to sit down at the same table with those known to take part in psychiatric repression. In any case it is necessary in the course of discussions and resolutions at any international meeting to speak openly to Soviet psychiatrists, saying all you think about the repression, and thus to extend the hand of help to the honest scientists who stand bashfully behind their dishonest colleagues. It is necessary to expand the participation of Western psychiatrists in the struggle for each victim of psychiatric repression. Only in this way, without and within, can one help cure Soviet psychiatry and establish in it such people as my comrade in prison, Semeon Gluzman. □

No season to be jolly

David Spurgeon reports from Ottawa on recent nuclear developments in Canada

CANADA'S nuclear programme has been in something of a turmoil lately. Certainly for officials of Atomic Energy of Canada Ltd. (AECL), the Crown corporation charged with nuclear R&D, exports and technical aid, the season has definitely not been jolly—quite the reverse. The opponents of nuclear power have stepped up their attacks in public, in AECL's eyes sometimes quite unfairly. The auditor-general has questioned their accounts. And that has led the House of Commons' Public Accounts Committee to investigate what looked like highly unusual and extraordinarily large payments to agents in such unlikely places as Panama and Liechtenstein; these had apparently been made as commissions on CANDU reactor sales to countries like Korea and Argentina, which many thought should never have been sold reactors at all.

The auditor-general's report then added insult to injury by revealing that AECL lost \$100 million on one of these deals, though it was later disclosed that this loss could be reduced to \$25 million by yet another sale now being negotiated with the same country, Argentina. It was shortly after all this appeared in front-page newspaper headlines that the Minister of External Affairs, Mr Donald Jamieson, announced a new nuclear export policy which because of its stringent requirements for safeguards will probably restrict overseas sales of reactors in the future.

This was less a direct result of the foregoing events, however, than a product of India's detonation of a nuclear device in May, 1973, with the help of a Canadian research reactor. But it has already had a paradoxical effect: since Pakistan would not agree to the terms of the new policy, Canada's nuclear aid programme with that country will be cut off, in spite of the earlier contention of the Prime Minister, Mr Trudeau, that Canada should not withhold nuclear technology from developing countries. Mr Jamieson has admitted to the press that there is a real possibility that the nuclear policy decision will cause electrical brownouts in Karachi.

To some people, all this seems to strengthen the suggestion made not long ago by John Shepherd, executive director of the Science Council of Canada, that AECL should give up its export sales programme of CANDU reactors and concentrate on exploiting

what could be a huge domestic market. To do this properly, he proposed that AECL should revert to its R&D function and help form a consortium with private nuclear industry and the provincial power companies.

Long and complicated

The story of the commissions on CANDU sales is a long and complicated one not yet revealed in its entirety to the Canadian public. One commission, on the Korean sale, amounted to \$18.5 million. The company involved, United Development Inc. (UDI), reported that it paid \$1.3 million of this to the Triangular Trading Company of Panama as a portion of the \$8.1 million it claimed for expenses in the deal.

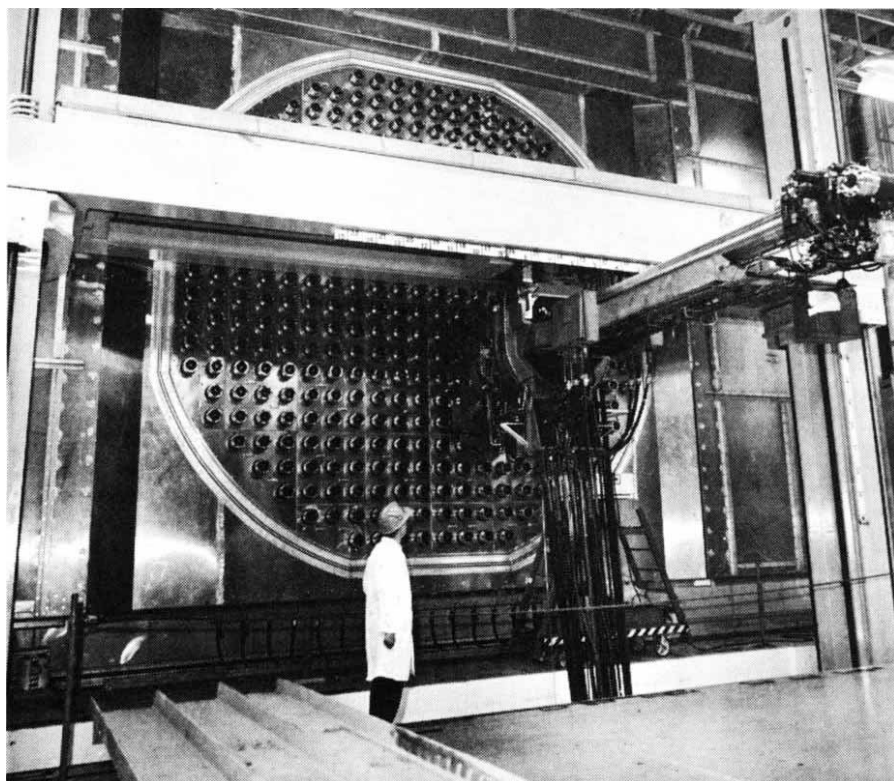
UDI, whose headquarters are also listed as being in Panama, also paid fees totalling more than \$1 million to firms in Hong Kong and Frankfurt for technical and economic studies of CANDU compared with other nuclear systems. AECL agreed to pay the fees after it had renegotiated the agent's contract and reduced the commission from about 5% to 1%, plus documented expenses.

UDI is headed by Shaul Eisenberg of Tel Aviv, and it was the inadequate

documentation for the \$8.1 million expense account paid to him that prompted the auditor-general, J. J. Macdonell, to report it to parliament. Mr Macdonell made it clear that AECL officials tried to persuade him not to make the matter public, and AECL's chairman, Ross Campbell, admitted this but said he was concerned not about the facts but about the innuendos that would damage AECL's reputation and those of the companies it dealt with. In fact, a special study of AECL's financial management on behalf of the auditor-general was strongly critical of its overall handling of expenditures. This study, unrelated to the Korean sale, was part of a continuing review of Crown corporation's management.

The second commission case involved a joint sale by AECL and Italimpianti of a CANDU reactor to Argentina. The Italian firm obtained an agent for this sale and then asked the Canadian government to pay half the cost. Canada responded by having \$2.4 million deposited into a Swiss bank account held by a trading firm in Liechtenstein, as requested, but never learned the name of the agent or whether it was the Liechtenstein firm.

The Italian firm stalled on providing the answer, moving the Canadian Energy Minister, Alastair Gillespie, to wonder publicly if "there may be Canadians involved." He said the Royal Canadian Mounted Police had been asked to find out who received the \$2.4 million, if they could.



Fuelling a CANDU reactor: no shutdown is needed

Exports restricted

The new nuclear policy announced by Mr Jamieson just before Christmas means that Canada will restrict its exports of nuclear equipment and materials to countries that either have signed and ratified the Nuclear Non-Proliferation Treaty or have "full-scope" nuclear safeguards. In addition, these countries will have to accept the kinds of additional bilateral safeguards announced by Canada two years ago. The alternative to adherence to the NPT means that a country must accept equivalent international safeguards on its complete nuclear programme (outside military aspects if it has nuclear weapons already). This is what Pakistan refused to do.

Announcing the policy at a press conference, Mr Jamieson claimed that Canada now has the toughest requirements imposed by any nation supplying nuclear materials. He said Canada decided on the policy only after it had failed to persuade other members of the so-called London Club to take similar steps together. Canada now hopes that these other nuclear-supplying countries will follow its lead, which is designed to prevent the proliferation of nuclear weapons. And he suggested that recent tightening of policies by the United States and France was a hopeful sign.

Canada's new policy is seen also as a reinforcement of a safeguards policy announced two years ago, which some uranium customers have not been observing closely. A number of countries, including Japan, Switzerland and the EEC countries, have not yet completed all the required safeguards. After allowing two six-month extensions of supplies, the Canadian government has announced that it will cease uranium shipments at the end of December, 1977, to force compliance with the policy. Mr Jamieson said he hoped that by that time the safeguards would be in place and supplies could continue uninterrupted.

In notes distributed before his press conference, Mr Jamieson said.

Nuclear export policy already requires binding assurances that what Canada provides will not be used for explosive purposes. Existing policy, however, does not cover what a country receives from other suppliers or what it might do on its own. The new policy will close this gap. We will have, therefore, assurance by treaty that Canada's nuclear customers will have been selected from amongst those countries which have made a clear and unequivocal commitment to the non-proliferation of nuclear weapons.

Mr Jamieson added that Canada recognises the legitimate energy requirements of its trading partners, but is determined to do all it can to avoid



Don Jamieson, External Affairs Minister

contributing to nuclear weapons proliferation. It was for that reason that Canada had unilaterally decided to strengthen further her safeguards requirements. As in the past, "We are prepared to accept the commercial consequences of being clearly ahead of other suppliers. This is the price we are prepared to pay to curb the threat to mankind of nuclear proliferation." □

Holland's nuclear problems

Casper Schuurin reviews a troubled year for nuclear power in the Netherlands

IF it had not been so before, nuclear energy became a major political topic in the Netherlands in 1976. The issue was alive as early as January when debates on the subject led to a government decision to postpone definite planning for three 1,000 MW nuclear power plants until after the next elections in May 1977. The Netherlands already has two existing smaller plants, of 447 MW and 54 MW.

Overcapacity in generating power and uncertainties regarding the nuclear fuel cycle were among the main reasons for the move, but political factors were important too. The small Radical Party (PPR), which takes part in the country's coalition government with Labour and Christian Democrats, has two ministers and an under-minister very much opposed to nuclear energy—one of whom is Fokele Trip, the Minister for Science Policy.

Labour, too, has doubts about nuclear power. The Christian Democrats are less perplexed. One of their

ministers, for Economic Affairs, had planned the three nuclear plants in his energy memorandum of September 1974. Construction of the first was to start in 1976. Following the government decision to postpone the building of the plants, a declaration signed by 1,200 scientists covering a range of disciplines expressed their concern at the rapid expansion proposed for nuclear energy—as 2,300 colleagues of theirs had done earlier in the United States. Published at the end of January and originally addressed to the government, the declaration was then directed to parliament, which was to discuss the memorandum in February. With the decision on additional nuclear plants pushed to the next government, however, the parliamentary discussions lacked excitement.

Calm doesn't last

The calm was not to last. A conflict soon arose over a credit guarantee to be given by the government for the

building and selling of components for two power plants in South Africa. The work was to be done by a group of industries participating in a consortium together with American and Swiss industries. The government had already indicated in mid-1975 that an export licence would be given, but in parliament a vote of censure was just passed covering exports of nuclear material and equipment to countries that had not signed the nuclear Non-Proliferation Treaty. South Africa is such a country.

The consortium was originally to have built two reactor vessels; but with a change in arrangements the vessels were to be built by General Electric and the Dutch industries could still take part in the work, for which only an export guarantee was then necessary. Discussion went on so long that at the end of May the deadline passed and a French consortium won the South African order. The Dutch government itself just survived a long and critical period, and relations between government and industry sustained a good deal of damage.

During the summer the Steering Group for Energy Research issued its second report. This concluded that conservation of energy should form a considerable part of Dutch policy, and

offered two scenarios for energy use over the next 25 years. One involved a continuing growth of 4½% until the year 2000, and the other a levelling off to zero growth around 1995. The latter scenario, which the Group itself backed, still involved building 6,500 MW of nuclear plant in 2000; the government and electricity producers were planning between 10,000 and 14,000 MW at that time (Holland now has a total generating capacity of 14,000 MW and plans 39,000–56,000 MW by 2000). The nuclear research centre was meanwhile transformed into a general energy research institute, incorporating work on wind, fusion and flywheel programmes; a national programme on solar research is foreshadowed for early this year.

Breeder intention

By last autumn the Economic Affairs Minister Mr Lubbers was informing parliament almost casually of the intention of provincial and local electricity producers to take part in Superphenix, France's first commercial fast breeder. Some time before, the minister had shown some antipathy towards continued Dutch cooperation with Belgium and Germany—the three countries are building the 300 MW SNR-1 in Kalkar and are each carrying out a part of the research.

In a letter to parliament at the end of August the minister confirmed that the Netherlands was in principle continuing with the Kalkar project until SNR-1 was finished, and that Belgium and Germany had agreed to make a decision on a commercial breeder (SNR-2) then. He added that the electricity producers were involved in the SNR project, and were also in the larger European group which will develop the breeder concept further. This group, he said, would proceed with the building of the 1,240 MW Superphenix, which is based on the Phénix, before the SNR-2; as a decision to build this would be taken in the beginning of October, the electricity producers had to decide on their role.

An urgent debate was held in parliament, mainly because the Radical and Labour parties thought the Netherlands would become involved in the commercial development of a breeder before some problems were solved and vital experience was gained. As a result of one of the votes in parliament, Dutch participation is only acceptable once very strict conditions are made regarding safety aspects and international control of the project. The electricity producers agreed, and accepted the maximum financial contribution imposed on the 4,000-million guilders project, though not without some disagreement amongst themselves. By the

end of the year, however, no decision to build the breeder had been taken.

Fourth time

When another nuclear subject attracted attention in October, it was for the fourth time in the year, and in this case created a problem lasting until close to Christmas. The problem concerned uranium enrichment using the gas centrifuge technique, and a definite solution is not actually expected before the end of February. The Anglo-Dutch-German group known as Urenco has pilot plants in Capenhurst in Britain and Almelo in Holland, and partly finished demonstration plants. The difficulty is that a decision is due on extensions to increase Almelo's capacity to 1,200 tonnes. Towards 1987 a further expansion for Urenco to 6,000–8,000 tonnes is foreseen, and the consortium has definite orders for 2,000 tonnes enriched uranium a year from 1983.

The Radicals are firmly against an expansion of the enrichment capacity for reasons connected with proliferation. There are also commercial objections, and these come not only from the PPR. Mr Lubbers has given confidential information to the parliamentary commissions for foreign, economic and nuclear affairs, but there is hesitation and doubts remain over both proliferation and the possibility of a costly over-capacity in the international enrichment market. The German-Brazilian deal is seen as particularly worrying. The Reflection Group on Energy Policy—active in 1975 before the decision on the three power plants was made—wrote a memorandum on the issue, and the PPR's own group of consultants published a report.

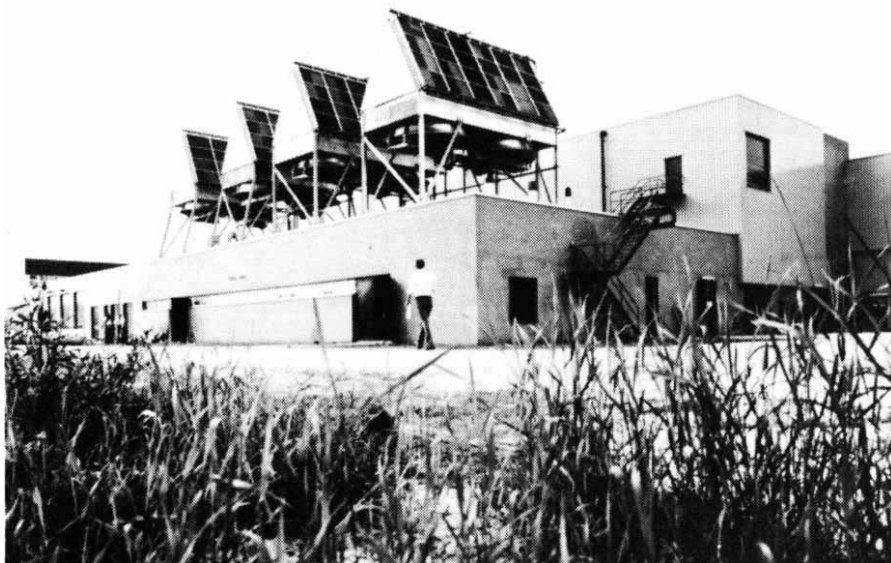
At the same time as all this happened the announcements from the

USA and France of restrictions on nuclear exports became known, as did details of the resistance in Germany to a rapid expansion in the number of nuclear power plants. A further important point was that large commercial concerns like Shell, Philips and DSM, originally involved in the project, did not like to invest more money in it for both economic and political reasons. They believe it is impossible to work when political aspects play such a great role in decision-making.

State of crisis

A state of crisis existed for several weeks in The Hague, but the cabinet finally decided to agree provisionally to participate in Urenco's expansion, which also involves the Capenhurst plant. The parliamentary committees were informed, but it is still not clear what precisely the Dutch ministers of economic and foreign affairs will discuss with their British and German colleagues on the matter. The decision could mean domestic political problems. The Radicals are still against the idea and could conceivably leave the government. Labour and the Christian Democrats, however, are not willing to export enriched uranium to countries which have not signed the NPT. Each member of the tripartite consortium has the right of veto according to the Treaty of Almelo of 1970, and in Holland the tendency now seems to be to have this exercised not only over the export of uranium but on all nuclear exports.

At the very least, whether this government breaks up over the issue or not, the nuclear power debate will prove important in the formation of the next government. Developments during 1976 have seen to that. □



Urenco's plant at Almelo, Holland

USA

Judgment of the people

Colin Norman reports from Cambridge, Massachusetts, on the recommendations of a citizens committee established last year to look at genetic manipulation experiments

IN a report likely to have important and widespread implications, a committee of citizens of Cambridge, Massachusetts, has recommended that experiments involving a new technique for manipulating genes in living organisms—recombinant DNA experiments—should be allowed to go ahead at Harvard and Massachusetts Institute of Technology. The committee said, however, that the experiments should be permitted only if they are placed under safety controls more strict than those now in force in most other institutions, and that a special board, consisting of Cambridge residents who have no affiliations with the two universities, should be established to monitor recombinant DNA research in the city.

The committee was set up last summer by the Cambridge City Council after a shrill and bitter dispute had erupted over plans to conduct recombinant DNA experiments at Harvard and MIT. At the urging of the city's colourful and irascible Mayor, Alfred E. Vellucci, the council also placed a three-month moratorium (later extended until January 7) on some types of recombinant DNA research in Cambridge while the committee conducted its investigation.

The report is far from being the last word on the matter, however. Even before the document had been formally presented to the council at a meeting held on January 5, Vellucci told a few reporters that he disagreed with the recommendation that the research should be allowed to proceed in the city, and he said that he will propose a resolution at a later council meeting that some types of recombinant DNA experiments be outlawed entirely in Cambridge. Though it is generally considered unlikely that Vellucci's view will prevail in the council, the shouting is clearly far from finished.

The committee's work and its final report have won qualified praise from both sides in the dispute and, if nothing else, it has provided ample support for the view that citizens groups are well qualified to consider and pass judgment on complex technical issues. The committee, which was chaired by Dan Hayes, a former Cambridge Mayor, was thrust into the

middle of a highly charged political and scientific conflict, and it has acquitted itself admirably.

Events in Cambridge are important in the context of national and international efforts to control recombinant DNA research. Those efforts began in 1974 when a group of scientists went public with their concerns that genetically modified microorganisms produced in recombinant DNA experiments might pose a hazard to public health if they should escape from the laboratory and survive in the environment. Last June, the National Institutes of Health (NIH) published a set of guidelines designed to guard against such a possibility. They define four levels of physical containment to be used for recombinant DNA experiments, designated P1 to P4, and the guidelines assign experiments to them on the basis of potential risk.

In addition, the guidelines specify that some experiments should only be carried out on crippled strains of microorganisms which have a limited capacity to survive outside the laboratory. The restrictions formally apply only to research supported by NIH, however, and they have encountered considerable criticism from opponents of the research.

While the guidelines were being drafted, plans were drawn up to construct a safety laboratory at Harvard at which some recombinant DNA research would be undertaken. Under the definition in the NIH guidelines, it would be a P3-level facility. A similar laboratory had already been constructed at MIT for cancer research, and it was also expected to house some recombinant DNA experiments. The proposed Harvard lab became a source of dispute between opponents and proponents of the research, and the conflict spilled over into the city council after an article was published in a local newspaper.

Town-gown relationships have never been very friendly in Cambridge, and Vellucci quickly latched on to the issue. After two extraordinary council meetings in late June and early July at which the warring scientific factions presented their views, the Council imposed a moratorium on P3- and P4-level experiments in Cambridge and asked the citizens' committee to recommend the circumstances, if any, under which such research should be permitted.

The committee's investigation is thus the first detailed review of the NIH guidelines to be carried out by a

group composed entirely of non-scientists. It should be emphasised, however, that it was asked to consider only P3 and P4 experiments, and thus it was not asked to consider the rationale used by NIH in assigning experiments to different safety levels. It was also asked to consider only the potential health hazards of the research and it therefore steered clear of such emotive issues as whether the research might lead to undesirable applications.

After conducting lengthy public hearings and poring through reams of written material, the committee reached the unanimous conclusion that P3-level experiments can be permitted in Cambridge under certain conditions. Since no P4 facility is planned in the city, the committee said nothing about P4-level experiments, though it implied that they should not be carried out there. The chief condition set by the committee is that all recombinant DNA experiments should be performed with strict adherence to the NIH guidelines, but it also recommended the following additional safeguards:

- All laboratory personnel should receive adequate training in safety procedures, and institutions planning to carry out such experiments should produce a manual of procedures.
- Under the NIH guidelines, biohazards committees must be established in institutions where recombinant DNA experiments are conducted, and the Cambridge committee recommends that they should contain technicians and at least one representative from outside the institution.
- All P3 level experiments conducted in Cambridge should use crippled strains of microorganisms.
- Institutions must conduct tests to ensure that experimental strains of organisms are pure and that they are not resistant to common antibiotics.
- Institutions in which recombinant DNA experiments are performed must "in good faith make every attempt, subject to the limitation of the available technology" to monitor for the escape of host organisms, and "this should include whatever means is available to monitor the intestinal flora of the laboratory worker".
- Finally, the committee's most interesting recommendation is that a Cambridge Biohazards Committee (CBC) be established "for the purpose of overseeing all recombinant DNA research that is conducted in the City of Cambridge". The Board would be composed of people appointed by the city manager and its members would have no affiliations with the universities. Its functions would include reviewing pro-

posals for recombinant DNA research to ensure that they would comply with the NIH guidelines, maintaining links with the biohazards committees at Harvard and MIT, and "developing a procedure for members of institutions where the research is carried out to report to the CBC violations either in technique or established policy".

In addition, the committee urged Congress to pass legislation extending the NIH guidelines to cover all recombinant DNA research in the United States and to set up a national registry of laboratory workers for long term epidemiological studies.

The report has been welcomed by supporters of the research. David Baltimore, a tumour virologist at MIT's Cancer Research Center, said in an interview last week that the report "is basically a vindication of the whole process which began with the NAS committee", which raised the first concerns about the potential hazards associated with the research.

Opponents of the research are dis-

appointed that the committee didn't come down in favour of banning the research in Cambridge, but they also agree with much of the report. In particular, Jonathan King, an MIT biologist who has been among the more outspoken opponents, noted in an interview that creation of the CBC would be a good step toward more citizen control over research and it would lead to increased accountability of scientists to the public. He also welcomed the suggestion that the health of laboratory workers be monitored, but suggested that unless pressed the universities would drag their feet in implementing the recommendation.

The ball is now back in the City Council's court. At the meeting on January 5 when the report was publicly unveiled, the council passed a resolution extending the moratorium on P3-level experiments in Cambridge for a further 30 days to allow time to consider the recommendations and to hear responses from the universities. Vellucci has promised to introduce a

resolution banning P3- and P4-level experiments entirely and making scientists who conduct such research liable for a fine of \$1000 per day. Another resolution will be proposed to implement the committee's report.

One Cambridge Councillor, David Clem, said last week that he expects the report to be well received, and that the matter will probably be cleared up before the extended moratorium is scheduled to end. He noted, however, that he has some reservations about the recommendations and will wait to see what plans the universities have for implementing them before he decides how to vote.

Finally, it should be noted that other local governments, particularly the New York Attorney General's office, are taking a close look at the control of recombinant DNA research. The fact that a committee of non-scientists has recommended in favour of going ahead with the research, albeit under strict controls, will not pass unnoticed. □

PAKISTAN

New policy on the anvil

Azim Kidwai reports from Karachi on changes expected in Pakistan's science policy framework

THE economic, social and cultural changes that have occurred under the Bhutto government—they include nationalisation of almost all domestic banks and insurance companies, of the heavy industrial and parts of the agricultural sector, but not consumer goods industries like textiles, tobacco, chemicals and pharmaceuticals—have naturally had an impact on science policy. A new national policy now on the anvil is the result of lengthy deliberations amongst the elite of the scientific community and representatives of various scientific organisations and government agencies. Final proposals are expected once they have been discussed at Cabinet level.

The existing arrangements for scientific and technological research in Pakistan appear to suffer from three weaknesses: coordination of effort at national level is lacking; the universities have a very weak base as far as scientific research goes; and no effective mechanism exists for the utilisation of the results of research. At the apex of the structure of science in Pakistan lies the Ministry of Science & Technology of the central government assisted by the National Science Council and the Pakistan Science Foundation.

The National Science Council was created as a coordinating body for the different Research Councils and to advise the government on promotion of science in general, but in practice rarely discharged those functions effectively, possibly because it had no financial or budgetary strings to pull. It thus remained but a shadow of what it was originally conceived to be. The Pakistan Science Foundation was created subsequently as a sponsoring body to help promote financial support for university research work and promising individual research workers, for pilot plant studies, for the establishment of science museums and for scientific societies. This it has been doing within the limits imposed by the funds at its disposal.

This triangle at the top lacked co-ordination, and in practice was not up to implementing a vigorous science policy fully integrated into the national development effort. Suitably informed decisions could not be made at the top political level, and administrative coordination was inadequate, particularly in respect of planning. The proposals for the new science policy try to take account of these weaknesses. A National Council for Science and Technology is proposed as the supreme body to provide guidance on all matters pertaining to formulation and implementation of government policy on science and technology. It should also be responsible for planning, coordination and cooperative imple-

mentation of scientific and technological programmes. The proposed National Council may be headed by the Prime Minister, and the central ministry for science is to act as its secretariat.

The Research Councils—the Pakistan Council of Scientific and Industrial Research, Pakistan Medical Research Council, Irrigation, Drainage and Flood Control Research Council, Council for Works and Housing Research, Agricultural Research Council and the recently approved Livestock Research Council—are likely to be given autonomous character and resources to organise mission-oriented research in their specialised fields.

Some idea of the scope of R&D effort through these institutions can be gained from the budget figures for the current year, which reflect to some extent their relative weaknesses and strength. These are shown in the accompanying table. □

Pakistan's science research budget 1976-77

Council	Rupees
Scientific & Technological Research Division of the Ministry	1,998,000
National Science Council	443,000
Pakistan Science Foundation	5,500,000
Pakistan Council of Scientific and Industrial Research	29,320,000
Pakistan Medical Research Council	1,506,000
Irrigation, Drainage and Flood Control Council	740,000
Council for Works and Housing Research	820,000

£1 = 16.5 Pakistani rupees

correspondence

The nuclear debate

SIR,—I believe *Nature* is right to enlarge the nuclear debate to the general, international scene but I wonder if both sides can be represented fairly. Döderlein's essay (November 18, page 202), which presents one side of the issue, needs to be balanced by these points:

- The battle is one-sided. Pro-nuclear propagandists are paid; the opposition is not. Most nuclear "experts" are employed by the nuclear industry, which has money to spend. For example, anti-nuclear forces were outspent four to eight or more times in the six states where initiatives were balloted in November (*Science*, November 19). Another disquieting factor is that nuclear industry representatives seem to want to exclude biologists, environmentalists and other concerned members of the general public from the controversy. This approach does not generate confidence.

- Your opponent is always "irrational", as I find Döderlein. I also object to his suggested dichotomy: "reasonable, rational and valid" (or "reasoned, factual and rational") versus "irrational, emotional and ethical". One wonders what Döderlein's definition of rational might be.

- In my opinion humanity should be glad certain persons "... play out some of their inner and emotional needs by taking an active part in the nuclear controversy ...". These "needs" may be a safety valve for society. Humans resist becoming robots, they have emotions and let us hope they will continue to learn to express them for our collective betterment. Also, "emotional" and "irrational" should not be confused; an emotional approach can be disciplined by rationality and even by the scientific method. Emotionlessness is not unjustly linked with death.

- The fact that already "... industry ... gives uncontrolled releases ... of substances which remain toxic for infinite times ...", if true, would hardly be an argument for accepting nuclear wastes. Furthermore, all toxic materials do not have the same toxicity and we are not obliged to make an unweighted choice among them.

- Practically all the plutonium present on earth, contrary to Döderlein's implication, has been produced by man-made nuclear reactors. No natural source of plutonium is known although

trace quantities have been reported in uranium deposits. Any plutonium that might have existed on the primitive earth (of 4.6×10^9 years ago) would have long since disappeared because of plutonium's relatively short half life.

- "Medical" reactors may be much smaller than the bomb-capacity commercial reactors planned, hence medical reactors and small research reactors are less directly linked to the spread of nuclear weapons. I believe many people, some nuclear experts included, would like to see *more* nuclear research. We are alarmed by the planned installation of hundreds of big reactors all over the earth without enough research having been done, especially on medical and genetic risks to nuclear workers and the general public. Some of these reactors will contain *tonnes* of plutonium (see Frank Barnaby, *The SIPRI Yearbook*, 1976). Inhaling a few microgrammes can kill you.

- Finally, Döderlein asks "... can anything short of actual war stop a determined sovereign nation from getting primitive but usable versions of any kind of contemporary weapons technology—nuclear, bacterial, chemical or any other?" I say war could lead to have-not nations getting nuclear and other modern killing techniques, especially if such countries align themselves with the "right" side in the conflict. In this context, both sides would probably be the right side. So, let us try something short of war.

It seems to me that instead of trying to restrict the discussion to experts we should indeed extend it to everyone. This would encourage a human synthesis, an overall approach on world problems that no one discipline or industry can provide. Our capacities for arriving at common solutions will obviously have to be developed. This could be helped by de-specialising scientific and philosophical methodologies, which could be combined and applied to questions such as disarmament, the energy and food crisis and nuclearisation of the earth. A convergence and interaction between science and philosophy is necessary for just solutions and a stable future world, if it is to be democratic.

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CERN'S project management

SIR,—In your article of November 25 marking the completion of the CERN 400 GeV SPS you paid rather less attention than might have been expected to the fact that the project has not only comfortably exceeded its original specification in several important respects, but has also been completed on time and within its budget. The excellence of CERN's project management is self-evident, but the matter cannot be left there.

It has been said that CERN is an exacting customer both technically and financially, because it takes great pains to define its requirements precisely and because it usually has a clear idea in advance of how much it will need to pay. On the other hand, it can be a cooperative if tough customer, providing technological expertise, advice and highly-qualified personnel to contractors who experience problems during production. The advantages to both sides in this close relationship are obvious, even though the efficient use of outside technological help may sometimes have to be imposed upon an unwilling firm.

Building the SPS has involved the development of working relations with hundreds of firms throughout Europe. Although CERN's contributions to advanced technology and the economic utility of its contracts have been fully documented^{1,2}, its influence as a good customer has perhaps been underestimated, if only because the immediate financial gains are almost impossible to quantify. Now that the customer-contractor principle is established as a fundamental concept behind much British research funding, it is essential to re-emphasise the point that the ultimate success of a complex scientific project depends as much upon the competence and scientific expertise of the customer as upon the performance of the contractor.

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¹ Meeting on Technology arising from High Energy Physics, Proceedings, CERN 75-9 (1974).
² Schmied, H., A Study of Economic Utility Resulting from CERN Contracts, CERN 75-5 (1975).

The Editor of *Nature* welcomes all correspondence. Letters should be kept as brief as possible.

news and views

Nations and numbers 1976

from Robert M. May

LAST year I gave a summary (*Nature*, **261**, 12; 1976) of the 1975 estimates of population magnitudes, growth rates, age structures, and patterns of urbanisation which were compiled for individual countries and for regional groupings by the Environmental Fund (1302 Eighteenth St, Washington, DC). Their 1976 wall-chart shows much the same figures, with the total world population swollen by about 94 million to an estimated 4,241 million, and still growing at around 2.2% per annum.

The many uncertainties in population estimates are perhaps best illustrated by the figures for the USA. The Environmental Fund gives the mid-1976 population to be 222.2 million, as opposed to the US Bureau of the Census figure of 215.7 million. The discrepancy arises because the official figure does not include the undercount of about 5.3 million which has been announced by the Bureau for its most recent census in 1970, nor does it include an estimated 1.2 million illegal immigrants (constituted of 800,000 who entered illegally and evaded deportation, and 400,000 from among the 11 million foreign visitors who entered legally during the past year).

Such uncertainties are writ large for China, which has issued no official figures since 1956. Estimates of the current Chinese birth rate range from

37 to 17 per 1,000. The Environmental Fund chart uses Aird's 'intermediate model' (Foreign Demographic Analysis Division, US Department of Commerce, 1975) for the population of China. This is substantially higher than the US Bureau of the Census estimate, whose world population figure is consequently 4,069 million. The UN estimate for China is yet lower; highest is the CIA estimate.

Overall, the UN estimates the world birth rate to be 33 per 1,000, one less than it was 20 years ago. The Environmental Fund chart shows 35 per 1,000. As available data are not accurate to better than 2 per 1,000, the exact number is anyone's guess.

The chart also summarises the number of acres of arable land per person (from the 1974 United Nations Food and Agriculture Organisation, *Production Yearbook*, **28**). Although there are considerable difficulties in definitions here, the trends are interesting: an average of 1.2 arable acres per person in Africa; 0.5 in Asia; 2.3 in North America; 0.8 in Latin America; 0.7 in Europe (0.3 in the United Kingdom); 2.2 in the USSR; 5.3 in Oceania. Among countries with more than 10 million inhabitants, low is 0.1 for Holland, Japan and South Korea, and high is 8.1 for Australia.

Of the 158 countries listed in the

chart, 35 were net exporters of cereal grain in 1948-1952; 18 remained in 1974 (*UNFAO Trade Yearbook*, **14**, 1960 and **28**, 1974). By regional grouping, the figures for net export of cereal grain, in millions of metric tons, 'then' (1948-1952) and 'now' (1974) are: Africa, -0.4 and -6.9; Asia, -5.8 and -47.1; North America, +22.5 and +76.4; Latin America, +1.2 and -0.4; Europe, -20.8 and -28.5 (United Kingdom, -6.9 and -7.3); USSR, +2.1 and +0.7; Oceania, +3.2 and +6.9.

These and other similar figures underlie the UN projections for annual growth in food supplies and in population until 1985 (see Murdoch and Oaten, *BioScience*, **25**, 561; 1975). For Africa, the projection is for 2.5% per annum growth in food supplies and 2.9% in population; for Asia (excluding China), 2.4% in food and 2.6% in people; for Latin America, 2.9% and 3.1%. Summing over all 'rich countries', the projections are for 2.8% per annum growth in food supplies and 0.9% in population; for 'poor countries', 2.6% and 2.7%. These trends are disturbing. They are made more so by the facts that the projections ignore rising energy costs, that a large fraction of the world population is malnourished at present and that the past UN projections have consistently erred towards optimism. □

Multiple controls in bacteria

from John Scaife

THE early choice of bacterial fermentation systems for gene control studies was a fortunate one. They prove to be excellent models of multiple gene control, readily analysed both *in vitro* and *in vivo*. The control of the lactose (*lac*) operon for example has two known components: a repressor protein and a protein termed CRP. The repressor, which directly governs the bacterium's response to β -galactosides, blocks the

binding of RNA polymerase to its site in the promoter where transcription is initiated. CRP actively facilitates this binding step (see Gilbert in *RNA Polymerase*, edit. by Losick, R., and Chamberlin, M., Cold Spring Harbor Laboratory, 1976) using 3', 5'-cyclic AMP as a cofactor. It thereby mediates in the *lac* operon the repressive effect of glucose (catabolite repression) to which many degradative operons are

subject. Glucose modulates the amount of cyclic AMP in the cell.

Within the *lac* promoter the CRP-binding sequence (Beckwith, Grodzicker, and Arditti, *J. molec. Biol.*, **69**, 155; 1972) has now been identified among promoter fragments *in vitro* (Majors, *Nature*, **256**, 672; 1975). It lies upstream from the polymerase binding site. Interestingly the normal polymerase site in *lac* deviates signifi-

cantly from other sequenced polymerase sites (Pribnow, *Proc. natn. Acad. Sci. U.S.A.*, **72**, 784; 1975). There is, however, a mutant, *UV5*, whose sequence is more in line with the others (see Gilbert, *op. cit.*). This strain does not require CRP for efficient *lac* function. The lesson is clear; CRP compensates for otherwise poor interactions between RNA polymerase and the normal *lac* promoter. How it compensates remains an unanswered question of great interest.

This well-founded and simple view of catabolite repression may nevertheless be incomplete. There is evidence that it might be mediated by more than one mechanism (Ullman, *Biochem. biophys. Res. Commun.*, **57**, 348; 1974). The behaviour of the galactose (*gal*) operon of *E. coli* has appeared particularly paradoxical. *In vitro* studies (Nissley, *et al.*, *J. biol. Chem.* **246**, 4671; 1971) clearly implicate CRP in *gal* function, yet mutants lacking CRP or cyclic AMP give good *gal* expression *in vivo* (Rothman-Denes, Hesse, and Epstein, *J. Bact.*, **114**, 1040; 1973).

New evidence for a second system mediating catabolite repression has just appeared. (Ullmann, Tillier, and Monod, *Proc. natn. Acad. Sci. U.S.A.*, **73**, 3476; 1976) have partially purified from *E. coli* a new factor (CMF) which represses catabolite-sensitive operons in growing bacteria. The factor could resolve some of the present anomalies. It is unlikely to act by degrading CRP or cyclic AMP. It could interfere with inducer uptake or affect transcription directly.

There is, however, a different explanation for the *gal* paradox. It is suggested by features of the newly elucidated *gal* promoter sequence (Musso, Di Lauro, Rosenberg, and de Crombrughe, *Proc. Natn. Acad. Sci. U.S.A.*, in the press). The *gal* operon has in its promoter binding sites for CRP and RNA polymerase like those of the *lac* operon. In *gal*, however, there are two start points for transcription. One of them uses CRP and cyclic AMP; the other does not, and in fact is inactive in their presence. Thus Rothman-Denes and coworkers were probably observing the CRP-independent mode of *gal* transcriptions.

Why should there be two transcription modes in this operon? The bacterium uses galactose in two quite different ways—as a carbon source and a cell-wall component—providing two metabolic contexts for *gal* enzyme synthesis. How they are distinguished can now be understood. Galactose as a carbon source induces the operon by inactivating the repressor. Musso *et al.* show that this requires transcription in the CRP mode, since the *gal* repressor hinders the binding of CRP, not polymerase. Without exogenous galactose, however, the operon must remain partially active to permit growth. This requirement can be satisfied by transcription, in the CRP-independent mode, despite the active repressor in the cell. The way is now open to tackle the challenging problem of how CRP-independent *gal* transcription is related to the growth process. Could it involve CMF? □

150 cm (PLT, 130 cm), the minor radius 39 cm (PLT, 40 cm). The toroidal field was operated at 3.5 T in both devices. The discharge current in these devices was 0.4 MA in T-10 and 0.59 MA in PLT. This current, which is in the toroidal direction, is essential for the formation of a plasma equilibrium and also heats the plasma by ohmic dissipation.

The parameters of the plasma contained in the two devices were broadly similar; the electron temperature in the centre of T-10 was 1.2 keV (1.9 keV in PLT), the ion temperature 0.6–0.8 keV (0.9 keV in PLT), the plasma density (n) $8 \times 10^{13} \text{ cm}^{-3}$ (3.5×10^{13} in PLT). The major difference between the two experiments was in plasma purity. In T-10 the plasma was rather pure having an effective charge between 1 and 2 whereas in PLT the effective charge was between 5 and 7. The energy containment time τ in T-10 was 0.06 s (0.04 s in PLT), giving an $n\tau$ (the Lawson product) of $5 \times 10^{12} \text{ cm}^{-3} \text{ s}$. Although the value of $n\tau$ and the ion temperature are both short of what is required in a reactor ($n\tau \sim 10^{14} \text{ cm}^{-3} \text{ s}$ and an ion temperature of 10 keV), they are a substantial improvement on the results obtained in smaller machines operated with the same magnetic field.

There was much discussion about the scaling of the energy confinement time τ between these larger experiments and the smaller experiments. The general consensus was that the energy containment time was scaling as the minor radius to the power α ($\tau \propto a^\alpha$) with α lying somewhere between 1.5 and 2. This result is quite encouraging and provided this type of scaling extends into the higher temperature region needed in a reactor, then it should be possible to achieve reactor conditions in a tokamak of modest size.

Moving on to the smaller tokamak experiments, the strong scaling of $n\tau$ with magnetic field strength has been demonstrated in the Alcator tokamak at MIT. Alcator is a small tokamak, major radius 54 cm, minor radius 9 cm, but has been operated with very high magnetic fields up to 8 T. At the highest magnetic fields, densities of $7 \times 10^{14} \text{ cm}^{-3}$ and containment times of 0.02 s have been achieved giving an $n\tau$ of $10^{13} \text{ cm}^{-3} \text{ s}$. Thus it seems that high magnetic fields have a significant effect on the plasma containment and allow operation at higher density.

The neutral injection heating of tokamaks has continued to be successful. Ion temperatures up to 2 keV have been obtained in TFR and Ormak, and the increase in ion temperature is proportional to the injected power. The ion energy confinement time seems to be independent of temperature and does not follow the neoclassical scaling

Fusion in Berchtesgaden

from J. G. Cordey

The sixth International Conference on Plasma Physics and Controlled Nuclear Fusion Research was held at Berchtesgaden in Bavaria on October 4–15, 1976. A more detailed account, which includes progress in fields of reactor design and inertial confinement (not discussed here) will be published soon in *Nuclear Fusion*.

DURING the past few years significant progress towards achieving thermonuclear fusion conditions has been made using the tokamak approach. This has been mainly due to the considerable effort that has gone into this field, which to some extent has been at the expense of the other containment

geometries. The plasma physics behaviour in a tokamak is not yet fully understood, but experimental scalings at present suggest that fusion conditions can be obtained in a modest sized experiment. As far as the other containment approaches are concerned the route to a reactor is less clear.

Since the last meeting in this series, held in Tokyo in 1974, several new experiments have been commissioned. In particular the first results from two new large toroidal experiments T-10 and PLT were presented. Both these experiments are of the tokamak type, PLT (Princeton Large Tokamak) is at the Princeton Institute of Plasma Physics in the USA and T-10 at the Kurchatov Institute in the USSR. The machines have similar dimensions, the major radius of the torus in T-10 is

The initial experiments on the DITE tokamak at Culham using the 'Bundle Divertor' are also quite encouraging. The purpose of a divertor is to remove impurities from the edge of the plasma. (Impurities poison the plasma and lower the temperature of the edge region.) The bundle divertor consists of two coils whose magnetic field distorts the main toroidal field such that the field lines at the edge of the plasma are diverted into an external pumping chamber before returning to the torus. The divertor on DITE is only operating at low toroidal field at the moment, but has successfully reduced the influx of gas and impurities to the plasma; as yet there has been no increase in electron temperature however.

Turning to other containment devices there was a revival of interest in stellerators at the conference. Results were presented from three new stellerator experiments—W7A at Garching, L2 in the Lebedev and Cleo at Culham. All these machines are smaller than the present generation of tokamaks, with minor radius of around 10 cm. Their energy containment time was typically between 1 and 8 ms with electron temperatures up to 800 eV. These results are clearly an advance over those obtained in the model C stellerator experiment at Princeton some years ago and are comparable with tokamaks of similar size and field strength. In fact these machines can be run in either a pure tokamak mode by not energising the stellerator helical windings or a stellerator-tokamak hybrid mode. Comparisons were made between the two types of operation and it was found that with the stellerator field on, the containment time was increased. Thus it seems that the extra complication of the stellerator helical winding can give worthwhile improvement in containment. I think, however, that the difficulty of designing suitable helical windings for large-scale devices with stronger magnetic fields may restrict progress in this field.

Both the tokamak and the stellarator are toroidal confinement devices and in these the magnetic field lines stay within the plasma volume. There is another

Tenth century map of the world exhibited at the International Geographical Congress in Paris. From *Nature* **15**, January 11, 234; 1877.

There has been a considerable increase in understanding the physics of mirror machine containment in the past 2 years, mainly due to experimental work on the 2XIIB machine at Livermore. Using very powerful neutral injectors delivering in total 5 MW of power at an energy of 20 keV, plasma densities of 10^{14} particles cm^{-3} at a mean ion energy of 13 keV have been built up from a target gas. To suppress the amplitude of ion cyclotron microinstabilities a warm plasma stream is injected along the field lines; unfortunately this stream also cools the electrons. In fact the electron temperature was less than 100 eV and this low electron temperature resulted in the rapid cooling of the injected ions; the energy containment time was typically of the order of 0.001 s giving an $n\tau$ of $4 \times 10^{10} \text{ cm}^{-3} \text{ s}$. The average value of β that is the ratio of plasma pressure to the magnetic pressure, was very high—of the order of 0.7. This may be compared with tokamak β values which are typically of the order of 0.01, so the mirror machine is very economic in the use of magnetic field. It is this, along with its simplicity, that makes the mirror machine attractive from the reactor designer's point of view.

plasma through the ends of the device. As well as losses caused by classical Coulomb collisions, there are additional losses due to microinstabilities which are driven by the anisotropy of the velocity distribution of the mirror-contained plasma. There are good theoretical arguments for expecting these microinstabilities to be less important in reactor scale devices with larger radius. But even if the only losses were classical, a self-sustaining mirror reactor would not be possible, and the device would have to be operated as an energy amplifier. Unfortunately the energy amplification factor Q is rather low, of order 2, and several schemes have been advanced for raising Q to around the 10 or 20 required for an economic mirror reactor. At this conference a novel triple mirror scheme was proposed by Dimov *et al.* (IAEA Conference Berchtesgaden paper C4). The idea is to place a small mirror machine at each end of a large mirror machine. The temperature and density in the outer mirror machines were maintained by high energy (~ 1 MeV) neutral injection at a higher level than the temperature and density of the main inner mirror machine. The resulting ambipolar potential of the outer mirror machines reflected the ions in the inner mirror thereby improving their containment. By suitably adjusting the ratio of the lengths of the central mirror machine to outer mirror machines, it was thought that Q values in the region of 5–10 could be obtained.

Fortunately, no basically new types of instability have been found during recent theoretical work and most of the theoretical papers were concerned

with analysing the effects of existing modes in more realistic geometry. There was also an interesting and welcome trend in that theoreticians were attempting to compare their predictions with experimental results.

The Oak Ridge theoretical group gave an analysis of the flux-conserved tokamak equilibrium. These equilibria are attained by increasing the plasma pressure in a time which is short compared with the magnetic field diffusion time. In this manner β values of the order of 0.3 are achieved. The current profile of this type of equilibrium has not yet been examined; nevertheless, if a tokamak can be operated at a high β then the reactor economics of the device will be greatly improved. $\square$

Environmental stress cracking

from P. D. Calvert

WHEN the bottom of a polyethylene bucket splits after six months use the cause is probably environmental stress cracking. The action of detergent solutions and residual stresses from the moulding process cause the slow growth of a crack which usually follows the boundaries of the polyethylene spherulites. Fifteen years ago this was a matter of great industrial concern as coverings on underground cables were prone to crack at bends. It was found, however, that the problem could be largely eliminated by suitable control of the grade of polymer and moulding conditions and the level of industrial interest decreased. But some progress has since been made on the more academic question of why this cracking occurs.

The phenomenon of environmental stress cracking has been extensively reviewed (see Howard, *Soc. Plastics Eng. J.*, 397; 1959; *Engineering Design for Plastics* (ed. Baer, E., Krieger, Basel; 1964)). Cracking is sensitive to the molecular weight distribution of the sample and in particular cracking resistance is improved by removal of the lowest molecular weight species (Herman and Biesenberger, *Polymer Engineering Sci.*, 6, 341; 1966), which probably segregate to the spherulite boundaries and weaken them. Tests for stress cracking measure the time to fracture of a sample either bent and immersed in a liquid, or held under constant load in the liquid.

High density polyethylene is more resistant than low density to stress cracking under a constant load, but the situation is reversed when bent samples are tested. Fire polishing improves re-

sistance to stress cracking, presumably by removing small surface flaws.

The liquids which cause stress cracking in polyethylene tend to be of low viscosity and to be surface active. Non-ionic detergents are quite effective, as are silicone oils, mineral oils and alcohols, but not ethylene glycol, glycerol or water. The liquid does not have to be a solvent or swelling agent for the polymer.

From a series of recent studies we now know that the mechanism of environmental stress cracking at low loads, involves void growth and depends on the ability of the liquid to penetrate from the sample surface to the crack tip.

In 1974 Hannon (*J. appl. Polymer Sci.*, 18, 3761) showed that the fracture surfaces consist of small smooth areas surrounded by zones where the polymer has been drawn out. He pointed out that this resembles the situation in ductile fracture surfaces of metals where fracture starts by the growth of voids around particulate inclusions and ends when the voids grow large enough to join up. In polyethylene the voids must start at locally weak regions. Addition of detergent to the testing environment results in larger voids with less deformed material. Berg (*J. Res. NBS*, 76C, 33; 1972) discussed theoretically the process of cavity growth in metals and suggested that this could be applied to stress cracking in polymers.

At high loads the crack velocity can become sufficiently large that the progress of the crack tip is limited by the ability of the liquid to flow in. On this basis Williams and Marshall (*Proc. R. Soc.*, A342, 55; 1975) made a fracture mechanics analysis of stress cracking and predicted a change in the stress dependence of crack speed, which they observed to occur at about $3 \times 10^{-7} \text{ m s}^{-1}$ in a detergent. Shanahan and Schultz (*J. Polymer Sci.*, (Phys), 14, 1567; 1976) have now established this change by measuring fracture times of polyethylene samples in a series of silicone oils of different viscosity. At low loads the time to failure is independent of viscosity but for each viscosity a critical rate is reached above which the failure time changes only slowly with load. This critical time to failure is roughly proportional to the fluid viscosity. The most obvious interpretation of this is that the total time to fracture is the time it takes the crack to grow, but Shanahan and Schultz make the rather more elaborate assumption that when, by creep, a critical strain of 15% is reached, fracture occurs rapidly at a rate proportional to the creep rate. This, however, is a point which ought to be easily cleared up.

Although this gives us a good idea of how the fracture occurs it does not

help to explain why polyethylene is the only polymer in which it happens and why some liquids are more effective than others. Spendal (*J. appl. Polymer Sci.*, 16, 2375; 1972) has shown that the addition of 10% rubber can dramatically improve stress crack resistance, presumably by toughening the boundary regions. With rather more subtlety Schonhorn *et al.* (*J. Polymer Sci. (Phys)*, 11, 1013; 1973) have shown that the addition of silanes to polyethylene can improve stress crack resistance and base their arguments on the ways in which the interfacial energies of polymer-polymer and polymer-liquid are modified.

There are many other cases where polymers do form voids under stress both with and without environmental agents; the phenomenon is generally called crazing. Why this should be so common amongst polymers and why only polyethylene is vulnerable to the peculiar form of voiding discussed above is not known although Haward (*The Physics of Glassy Polymers* Applied Science, 1975) has discussed the question in some detail. But the solution to this problem is unlikely to help the humble plastic bucket. $\square$

Serpentinite in the oceanic crust?

from Peter J. Smith

It is now more than 14 years since Hess (in *Petrologic Studies*, Geological Society of America, 1962) suggested that serpentinised peridotite is the major constituent of seismic layer 3 of the oceanic crust. In the debate that has gone on throughout most of the intervening period, arguments have been adduced both for and against the proposal, although the balance of belief now seems to be against it. As the serpentinisation of peridotite can only occur below 500 °C, Hess envisaged the hydration of upper mantle peridotite taking place upon the latter's moving upwards through the 500 °C isotherm beneath an oceanic ridge; but as Fowler (*Geophys. J.*, 47, 459; 1976) has recently pointed out, the depth and proximity of layer 3 to the ridge axis combined with a reasonable thermal model of the crust militate against widespread serpentinisation. Moreover, as Cann (*Geophys. J.*, 15, 321; 1968) has noted, the very small scatter of layer 3 seismic velocities would imply an unacceptably high uniformity of serpentinisation throughout the layer.

Cann's alternative suggestion is that the lower oceanic crust consists largely of gabbros and metagabbros. But even so, it does not follow that serpentinite

plays no part at all in crustal formation. On the contrary, it is evident that serpentinite bodies are closely associated with the major tectonically active fracture zones offsetting ridge axes. For example, the steep south wall of the Vema fracture zone which offsets the mid-Atlantic ridge by 320 km at 11°N is the edge of a serpentinite intrusion running parallel to the fracture and thus orthogonal to the ridge. And similar bodies, probably emplaced diapirically from the upper mantle, are also found in conjunction with offsets of the Indian ridge.

But serpentinisation in association with major fractures is a special case in that it is localised. Does serpentinised peridotite play any significant part in the formation of 'normal' oceanic crust along ridges? Bonatti (*Earth planet. Sci. Lett.*, **32**, 107; 1976) now suggests that it does—not in the form of a continuous layer *à la* Hess but as vertical solid intrusions, or 'protrusions', and associated sub-horizontal sills. The proposal here is that serpentinite bodies resulting from the hydration of upper mantle peridotite "move gradually upwards into the crust by solid diapirism during the initial stages of crustal spreading, and become emplaced along narrow bands parallel to ridge axes". By 'narrow' Bonatti means of the order of a few hundred metres; and the bands lie between the supposedly faulted blocks of basalt (layer 2) and gabbros/meta-gabbros (layer 3) which make up the bulk of the crust.

There is certainly evidence for the presence of serpentinite in the oceanic crust away from major fracture zones, at least in the Atlantic. For example, Aumento and his colleagues (see Aumento and Loubat, *Can. J. Earth Sci.*, **8**, 631; 1971) have reported that serpentinite outcrops are common near the mid-Atlantic ridge at 45°N. Numerous serpentinite samples have also been dredged from the north wall of the Vema fracture at 11°N. The latter, being found close to a major fracture, could be the result of fracture zone volcanism and thus irrelevant to the point at issue, although Bonatti offers convincing evidence for regarding the north wall of the Vema fracture (in contrast to the south wall) as a section of 'normal' crust. In any event, serpentinite samples from both sites share characteristics: they are restricted to no single crustal layer, they become shallower with distance from the ridge axis and they do not occur within a few tens of kilometres of the axis.

All of which would seem to support the idea of serpentinite diapirism. As Bonatti envisages it, the serpentinisation is due to sea water which both acts as hydrating agent and keeps the lower crust below the 500 °C isotherm.

The process begins at the fractured margins of the ridge axial zone and extends laterally away from the ridge for perhaps 100–200 km. Vertically, it occurs to depths of several kilometres, probably even down to the crust-mantle boundary. The upward mobility of serpentinised mantle is made possible by the existence of deep dip-slip faults parallel to the ridge axis (the result of block faulting) and the low strength and density of serpentinite compared with other crustal rocks. Finally, assuming a ridge spreading velocity of 1.2 cm yr⁻¹, Bonatti estimates the rate of ascent of the serpentinite diapirs to be about 1 mm yr⁻¹.

The serpentinite diapirs proposed are too narrow to be detectable seismically, but there may be other direct and indirect ways of deducing their presence. For example, serpentinites often have high magnetisations, probably due to the formation of secondary magnetite, and thus give rise to strong magnetic anomalies. If the Bonatti thesis is correct his oceanic diapirs should give rise to narrow anomalies superimposed upon the wider Vine-Matthews anomalies; and since the diapirs supposedly enter crust already thousands to millions of years old, their magnetic polarity should not necessarily agree with that of the surrounding crust. In fact, short-wavelengths linear anomalies of the suggested type have already been reported by several workers although unfortunately there are other equally possible explanations for them.

Perhaps the best test, however, is direct sampling. Serpentinite bodies in the oceanic crust have already been encountered on several occasions during the work of the Deep-Sea Drilling Project. Sub-parallel strips of intrusive serpentinised ultramafics of the order of 100 m wide have also been found in gabbros and basalts in a part of Macquarie Island believed to be a section of uplifted Cenozoic oceanic crust. There must surely be other examples.

Energetic nuclear collisions

from P. E. Hodgson

WHEN two nuclei collide at high energies there is a very strong interaction and many complicated reactions take place. The nuclei can excite each other and exchange a few nucleons before separating again, or they can fuse together for a short while to form a compound system that decays by emission of nucleons and alpha particles. At higher energies a nuclear shock wave can develop (*Nature*, **258**, 570; 1975) and this surges rapidly through the nucleus and causes the

emission of a spray of nuclear fragments.

Some progress has already been made in understanding these reactions using the classical concepts of viscosity and the mechanics of shock waves, but further progress requires a more detailed quantum-mechanical treatment. The motion of the nuclei must be described in terms of the coordinates of their constituent nucleons by wavefunctions satisfying the Schrodinger equation.

Many theories of nuclear structure have now been developed that treat the motion of the constituent nucleons in this way, in particular the Hartree-Fock self-consistent field theory. This gives a series of coupled equations for the nucleon wavefunctions that can be solved in principle to give the single-particle wavefunctions and all the properties that can be derived therefrom. This is a very complicated calculation that can only be carried out in practice by making several simplifying approximations.

But the problem of understanding a nuclear collision in a similar way is immensely more complicated, essentially because it is dynamic. Although a time-dependent Hartree-Fock theory has been developed, it is difficult to apply it to nuclear collisions.

Faced with such a complicated problem, the best way to proceed is to make a simple model that, it is hoped, contains many of its main features, but is practicable to solve. Comparison between the solutions of the simple problem and the experimental data will then indicate the usefulness of the model, and possibly suggest ways of improving it. At the same time increasing familiarity with the mathematical structure of the simple solution may make it easier to see how the model can be made more realistic without at the same time making it impossible to solve.

This approach has recently been used by Bonche, Koonin and Negele (*Phys. Rev.*, **13**, 1226; 1976) in a first attempt to make a quantum-mechanical theory of the energetic interactions of heavy ions. To make the calculations tractable, they made very drastic assumptions, and yet rather surprisingly the results showed many of the features of the experimental data and thus encourage the development of the theory in more detail.

The first great simplification was to assume that the whole interaction essentially takes place in one dimension. They thus considered the collision of two slabs of nuclear matter having a specified thickness in their direction of motion but extending indefinitely in the directions at right angles. They also assumed that these slabs were completely symmetric in spin and isospin.

The Hartree-Fock theory requires the interaction between two nucleons, and this is known to be very complicated. They therefore constructed a simplified form of the interaction that contains most of the physics and adjusted its parameters to give the best overall agreement with nuclear properties.

In its full generality, the time-dependent Hartree-Fock theory gives a set of coupled non-linear integro-differential equations in three dimensions for the wavefunctions of the nucleons. After making the above approximations they reduce to a set of coupled linear equations that are readily solved by existing electronic computer techniques.

These equations were solved for a series of different initial conditions. They first of all studied the oscillations of a single slab, and found that these could take place at certain definite frequencies. These oscillations correspond to the collective 'breathing mode' oscillations of nuclei, that is oscillations without change of shape. The frequencies of such oscillations depend on the nuclear compressibility (see *Nature*, **254**, 480; 1975).

Next they found out how a slab interacts with a potential, and in particular how it is affected by passing through a potential barrier. At a speed $E/A = 10$ MeV and barrier of Gaussian form and height equal to the incident kinetic energy there is hardly any reflection, and the transmitted slab has a different density profile showing that it is in an excited state. The excitation consists of density ripples moving through the slab, and calculations show that 36% of the incident kinetic energy is converted into such excitations. This behaviour is markedly different from that of a single particle encountering a barrier at comparable energies: in that case only half the incident wave is transmitted and half is reflected. This comparison shows the importance of collective effects in the slab interaction: the attractive single-particle potential due to the other nucleons moving

through the barrier reduces the effective potential and hence favours transmission over reflection.

As the barrier height is increased, so is the proportion of the slab that is reflected, until when the barrier is twice the incident kinetic energy it reaches 71%. In addition, the transmitted and reflected portions each split up into two pieces, one slowly moving and the other rapidly moving.

These studies are a useful preliminary to the main object of the calculations, namely to calculate the interaction of two nuclei, here represented by two slabs. At low energies ($E/A \approx 0.5$ MeV) the slabs fuse together to form a compound slab of rather uniform density that continues to oscillate without breaking up (see *Nature*, **261**, 193; 1976).

At a somewhat higher energy ($E/A \approx 3.5$ MeV) the compound slab is formed and then almost immediately separates again. Each fragment is oscillating strongly after the interaction, showing that it is highly excited. This process corresponds to the deep inelastic scattering of heavy ions, when two nuclei are strongly excited in a collision but essentially retain their identity, apart perhaps from the exchange of a few nucleons (see *Nature*, **256**, 261; 1975).

At much higher energies ($E/A = 25$ MeV) some quite new phenomena appear. As shown in Fig. 1, a region of very high density is formed, and from this region shock waves propagate outwards in both directions: these are indicated by the waves moving outwards at velocities higher than the incident slabs. When these waves of higher density reach the surface of the slab they separate from it, forming separate fragments that propagate rapidly outwards. Secondary waves follow them, and the region of almost uniform density that is formed for a short time in the central region breaks up from both ends into separate waves that also travel outwards and separate when they reach the surface, until eventually the whole compound

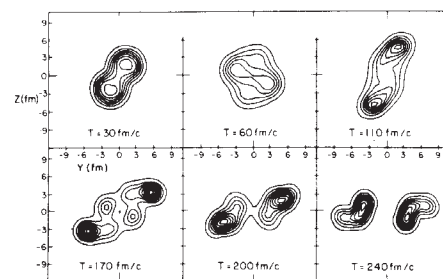


Fig. 2 Contours of the nuclear density for the collision of ^{16}O with ^{16}O at $E/A = 24$ MeV and an impact parameter of 4 fm. The contours are in the scattering plane and the density is integrated over the co-ordinate perpendicular to it.

system has broken up into fragments. Thus the model shows very well the phenomena of shock waves and fragmentation that take place in very energetic nuclear collisions.

An important advance has recently been made by Gusson, Smith and Maruhn (*Phys. Rev. Lett.*, **36**, 1166; 1976) in the form of a time-dependent Hartree-Fock (TDHF) calculation of the reaction $^{16}\text{O} + ^{16}\text{O}$ in three dimensions. The TDHF model they used assumes that the time-dependent wave function is given by a single Slater determinant whose occupied single-particle orbits obey the TDHF Schrödinger equation. For the interaction between the particles they used a simplified form of the Skyrme interaction, and assumed spin and isospin saturation, so that the spatial wave function applies to alpha particles.

Some of their results are shown in Figure 2, which gives the contours of the densities of the two interacting ions at various times in the plane of the collision, integrated over the direction perpendicular to that plane. The time scale is in units of fm/c, that is, about 3×10^{-24} s so the whole interaction takes about 10^{-21} s. These contour plots show that when a collision takes place at an impact parameter equal to about half the radius of the ions the outer regions interact strongly and stick the ions together in a dumb-bell shape which rotates about its centre of mass. As the system rotates, complicated internal motions take place, some parts rotating more rapidly than others. At one stage ($T = 170$ fm/c) small regions of higher density corresponding to alpha particles form in the region between the main mass concentrations. These are later attracted back and a neck forms between the two ions. The neck narrows and finally breaks, and the particles separate. They are now irregular in shape, indicating that they have been highly excited by the collision.

At no time in this interaction are high densities observed, indicating that the energy is too low for compression and shock wave phenomena to develop.

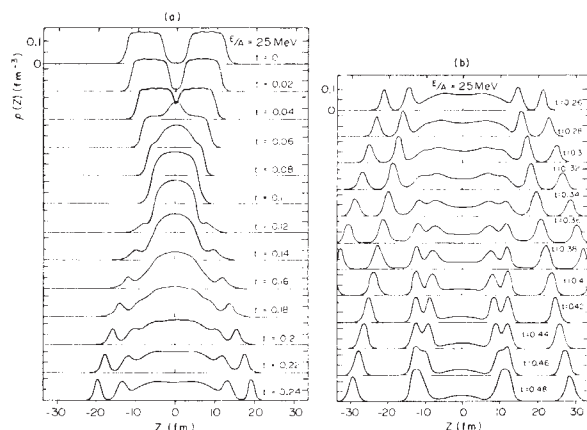


Fig. 1 Density profiles at various times t for the collisions of two slabs at a velocity corresponding to $E/A = 25$ MeV, showing the formation of a shock wave that propagates rapidly outwards, followed by a general fragmentation. (Times in units of 10^{-21} s.)

articles

New data on the lower Palaeozoic sequence of northern Victoria Land, Antarctica, and its significance for Australian–Antarctic relations in the Palaeozoic

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New stratigraphical and palaeontological data from the Early Palaeozoic Bowers Group of northern Victoria Land indicates that its depositional basin was probably closely linked with the Dundas Trough of western Tasmania. On the basis of these data, a revised reassembly of the Australo–Antarctic portion of Gondwanaland is proposed.

NORTHERN Victoria Land is a critical element in the reconstruction of the south-western Pacific sector of Gondwanaland. Most recent reconstructions of this sector have been based either largely on morphological best fits of continental margins^{1–6} or on 'plate tectonic' reconstructions, that is, by 'reversing' seafloor spreading using a mapped pattern of magnetic lineations and fracture zones^{7–11}. Only superficial attention has generally been given to the geology of the areas thus juxtaposed, primarily because of the lack of precise data from Antarctica.

Detailed evidence of distinctive structural–sedimentary belts that cross the Antarctic continental margin and thus serve as potential 'geological ties' has been singularly lacking, although broad correlation of Early Palaeozoic sequences has been attempted^{2, 4, 7, 12}.

This note presents new stratigraphical, sedimentological and palaeontological data from northern Victoria Land which are inferred to provide such a tie in the Cambrian–Ordovician, linking the Bowers Group depocentre in Antarctica with the Dundas Trough of Tasmania. Data from Lower Palaeozoic sequences elsewhere in Antarctica and from the Adelaide Geosyncline support a continental reassembly constructed on this basis, and further suggest that Cambrian sedimentation in this segment of Gondwanaland may have occurred in separate eastern and western belts of contrasting environment. It seems that the widely accepted 'computer fit' of Australia and Antarctica¹ is unlikely to hold, at least during the early Palaeozoic.

Bowers Group sequence, northern Victoria Land

All known Lower Palaeozoic sedimentary rocks of northern Victoria Land are included within the Bowers Group¹³, which lies within a 20–25-km wide infaulted belt extending south-east from near the mouth of Rennick Glacier for at least 270 km. To the north-east the Bowers Group rocks are inferred to be faulted against sediments of the Robertson Bay Group of probable Late

Precambrian age¹⁴; and to the south-west they are faulted against metamorphic rocks of the Precambrian Wilson Group.

The oldest of the three units comprising the Bowers Group is the Sledgers Formation, a dominantly marine sequence whose base is not exposed. The formation, of minimum thickness 3,000 m, consists of mainly angular unsorted basaltic breccia containing common fragments of pillow lava, and a few thin flow units in the north; to the south it interfingers with dark mudstone containing thin interbedded rippled fine sandstone and lenticular turbidite units of graded coarse volcanogenic sandstone and conglomerate¹⁵. The sequence has yielded no age-diagnostic shelly fossils.

Overlying the Sledgers Formation without apparent discordance is the Mariner Formation, a fossiliferous marine sequence comprising sandstone at the base and passing upwards into shale with thin limestone and sandstone beds. The youngest beds of the Mariner Formation are red sandstone and mudstone with desiccation cracks, rare inarticulate brachiopods and abundant trace fossils, including *Rusophycus* and *Cruziana*. This part of the sequence has been inferred¹⁶ to represent a marine regression, the youngest beds representing an intertidal environment. Shelly fossils (trilobites and brachiopods) are relatively common throughout the Mariner Formation, the basal beds being of late Middle Cambrian age (*Ptychagnostus nathorsti* Zone or *Lejopyge laevigata* Zone) and the youngest fossils being of middle or late Late Cambrian age. At the southernmost limits of known exposure the formation is at least 1,600 m thick, but in the north of the mapped area, where the upper beds have been removed by Late Cambrian erosion, it is only 100 m thick.

A regional unconformity truncates the Mariner Formation, the overlying fluvial deposits of the youngest unit of the Bowers Group resting with marked erosion but no apparent angular discordance on middle to late Late Cambrian intertidal deposits in the upper Mariner Glacier region, and in places with marked discordance on early Late Cambrian marine mudstone 170 km to the north¹⁷. This youngest unit is exposed in two separate strips forming the eastern and western margins of the Bowers Group outcrop. In the eastern strip at least 3,000 m of red-brown or yellow quartzose sandstone, with minor quartzose conglomerate and mudstone (Camp Ridge Quartzite) is exposed in the core of a major syncline. The sequence is dominantly fluvial and lacks body fossils, although locally near the base trace fossils including *Rusophycus*, *Daedalus*, *Monocraterion*, and *Skolithos* occur¹⁴. In the western strip of outcrop, mainly fluvial deposits of minimum thickness 800 m dominated by conglomerate (Carrier Conglomerate) occupy a similar stratigraphic position unconformably above fossiliferous marine beds¹⁵.

Late Cambrian and Early Ordovician granitic rocks crop out extensively to the west of the Bowers Group, but none are known to intrude it. Post-tectonic granitic plutons of Devonian to Early Carboniferous age (Admiralty Intrusives) intrude both the Robertson Bay and Bowers Groups. This igneous province is restricted to the area east of the Rennick Glacier, and is thought by some writers to be associated with tectonic movements (Borchgrevink Orogeny) which resulted in folding of both sedimentary groups^{13,18}.

Dundas Trough Sequence, Tasmania

The main locus of Cambrian sedimentation in Tasmania (the Dundas and Fossey Mountain Troughs) lay between the Precambrian Rocky Cape and Tyennan crustal blocks and the Forth Nucleus, forming an arcuate belt around the northern and western margins of the Tyennan Geanticline^{19,20}. The axial portion of the Dundas Trough contains two main marine sedimentary sequences separated by an inferred unconformity²¹. The older sequence, which contains no shelly fossils, consists of greywacke, siltstone, shale and quartzite with some interbedded mafic volcanics (Crimson Creek Argillite, Success Creek Group, and correlatives^{22–24}). Ultramafic complexes are found associated with these older rocks at several localities within the Dundas Trough, but are not known to intrude the fossiliferous Cambrian sequences.

The younger sequence comprises turbidite greywacke, siltstone, shale and conglomerate (Dundas Group and correlatives). It contains fossils (mainly trilobites) at several horizons; the oldest beds are Middle Cambrian (*Ptychagnostus gibbus* Zone) whereas the youngest beds range in age from early Late Cambrian (*Mindyallan* Stage) in the north²⁵, to late Late Cambrian (*Ptychaspis-Prosaikia* Zone) in the south.

Sediments of Middle to Late Cambrian age were also deposited in the Smithton Basin to the north-west of the Rocky Cape Geanticline²⁰, in the Dial Range Trough (a northern extension of the main Dundas Trough), and in the Adamsfield Trough to the east of the Tyennan Geanticline^{26,27}.

Along the eastern margin of the Dundas Trough, adjacent to the Tyennan Precambrian block is an arcuate belt of acid to intermediate volcanic rocks and associated sediments—the Mount Read Volcanic Arc. Rocks of the lower part of the sequence (Queenstown Pyroclastics, Central Lava Belt and Intrusives) grade westwards into greywackes of the Dundas Trough. The upper part of the sequence (Tyndall Group) unconformably overlies the lower part and is composed of tuff, agglomerate, sandstone and conglomerate; fossils of late Middle or early Late Cambrian age indicate that the Tyndall Group is, at least in part, of equivalent age to the Dundas Group to the west.

The Cambrian Dundas Group and Tyndall Group sediments are both unconformably overlain by the Owen Conglomerate or its equivalent, representing the basal unit of the Junee Group²¹. This contact marks a conspicuous change in lithology and sedimentation. The Cambrian units are predominantly of the siltstone–greywacke–greywacke conglomerate association, whereas the Owen Conglomerate and its equivalents are quartz- and chert-rich sandstone and conglomerate. Fossils of early Ordovician age are known from the middle part of the Junee Group and the unfossiliferous basal beds are likely to be of late Cambrian or earliest Ordovician age. Along the axis of the Dundas Trough contact between conglomerate and underlying beds is generally paraconformable²⁵; however, successively younger beds underlie the contact from north to south, so that at Birch Inlet the most southerly sequence—only a short break in sedimentation is inferred, whereas in the Dial Range Trough in the north, most of the late Cambrian is missing²⁵. Near the northern margin of the Tyennan Geanticline three small granitic plutons of probable Late Cambrian age²⁸ intrude Cambrian and older rocks.

Shallow water platform sedimentation continued over much of eastern Tasmania from Ordovician to Early Devonian times without notable interruption until a widespread period of deformation between the Early and Late Middle Devonian, known as the Tabberabberan Orogeny, affected all the Early Palaeozoic

rocks of the area. The Orogeny was accompanied and followed by granitic intrusions.

Cambrian sequences elsewhere

In Victoria, widely scattered outcrops contain sparsely fossiliferous Middle and Upper Cambrian shale and sandstone overlying an unfossiliferous sequence of spilites and cherts²⁹. Unlike the intensively studied Ordovician, however, the Cambrian sequences are poorly exposed and the stratigraphic relationships not sufficiently well known to be discussed in detail here.

In South Australia Early Cambrian rocks rest disconformably on Late Precambrian sediments in the main area of sedimentation, the Adelaide Geosyncline³⁰. The Lower Cambrian is characterised by a thick succession of shallow water shales and archaeocyathine-bearing carbonates. Extrusive igneous rocks are limited to a few small areas of basic to intermediate volcanics in the southern part of the Adelaide Geosyncline³¹, and some thin tuffaceous horizons within the Cambrian sediments of the Flinders Ranges³². In the late Early Cambrian, uplift led to erosion of older Cambrian sediments in the Yorke Peninsula area and the formation of conglomerates on both Yorke Peninsula and Kangaroo Island³². Widespread carbonate deposition at the beginning of the Middle Cambrian was followed by red-bed deposition which may have extended up into the Late Cambrian in much of the Adelaide Geosyncline, including Yorke Peninsula³², as fossiliferous late Middle and Upper Cambrian sediments are known from the Frome Embayment and the Warburton Basin further to the north.

In the Kanmantoo Trough, lying to the east of the Adelaide Geosyncline, a thick succession of metasediments (Kanmantoo Group) conformably overlies fossiliferous Early Cambrian sediments (Normanville Group). An inarticulate brachiopod, *Lingulella*, is found near the base of the Kanmantoo Group; however, with this exception, worm burrows are the only fossils known. The clear-cut contact between the Normanville and Kanmantoo Groups indicates the start of a new depositional cycle which was triggered by the Kangarooian Movements. These movements which affected the Fleurieu Peninsula, Kangaroo Island and Yorke Peninsula areas, lasted at least into the Middle Cambrian³³. Late Cambrian and Early Ordovician granites intrude the Kanmantoo Group in several places, thus placing a maximum age range of Early to Late Cambrian on its time of deposition. Other granites of similar age are found in southern South Australia. A folding movement (Delamerian Orogeny) took place in the Late Cambrian–Early Ordovician³⁴.

In Antarctica, the nearest known fossiliferous Cambrian sediments to those in northern Victoria Land lie 1,000 km to the south in the region between the Nimrod and Byrd Glaciers. Here the Byrd Group³⁵ comprises over 8,000 m of limestones with minor shale, sandstone, quartzite, and conglomerate unconformably overlying Late Precambrian metasediments. Archaeocyathinae occur sporadically throughout most of the sequence, but have been positively dated only from immediately north of the Nimrod Glacier. Here a small collection from beds within 1,000 m to 1,300 m of the base of the Group belong to the Lower Lena Stage (late Early Cambrian)³⁶. The stratigraphically highest Archaeocyathinae occur at least 6,500 m above the base of the Byrd Group, and are likely to be early Middle Cambrian.

South of the Nimrod Glacier widely separated outcrops of mixed clastic and carbonate sediments containing fossils of Early and Middle Cambrian age occur in the Shackleton Glacier³⁷ and Leverett Glacier³⁸ areas respectively. North of the Byrd Glacier, in south Victoria Land, scattered areas of unfossiliferous but probably in part Cambrian mixed carbonate–clastic sequences are also known³⁹. No further outcrops of calcareous rocks correlatable with the Early and Middle Cambrian Byrd Group occur between south Victoria Land and the northern coast, but a hint that the archaeocyathine limestone belt, now eroded or covered by ice, may once have extended as far north as the coast west of northern Victoria Land, is given by the record of marble erratics, one containing a possible Archaeocyathid, at Cape Denison⁴⁰ (Fig. 1).

Early Ordovician granitic rocks, which intrude the Byrd Group

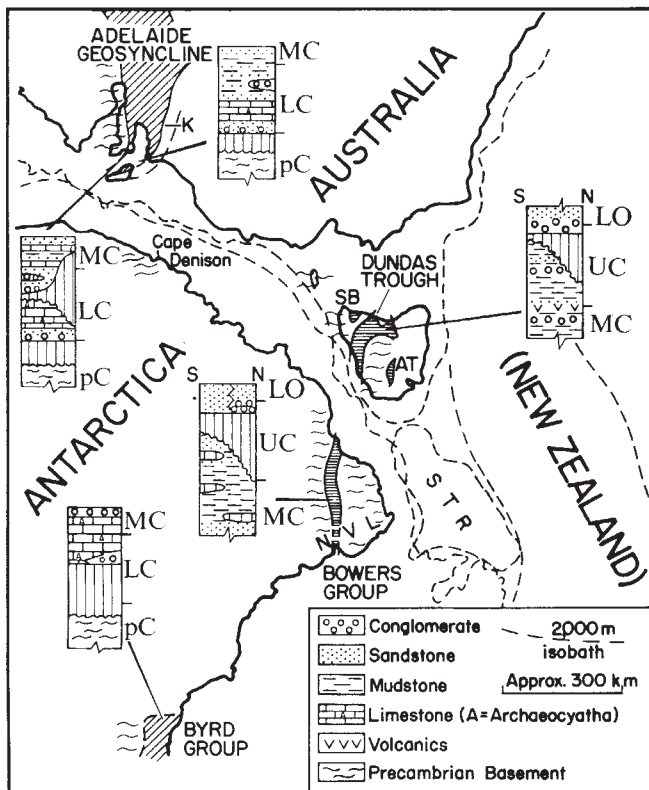


Fig. 1 Reconstruction of the south-eastern Australian and East Antarctic elements of Gondwanaland, showing the main Cambrian depositional sites (horizontally and obliquely hatched areas) and stratigraphic columns for critical successions. NVL, Northern Victoria Land; STR, South Tasman Rise; AT, Adamsfield Trough; K, Kanmantoo Trough; SB, Smithton Basin. Vertically hatched areas on the stratigraphic columns represent time gaps. Note: stratigraphic column for Dundas Trough is for the axial part, south to north, and thus does not include Mount Read Volcanics. The column for the Bowers Group similarly spans the belt from south to north. pC, Precambrian; LC, MC, UC, respectively Lower, Middle and Upper Cambrian; LO, Lower Ordovician.

south of the Nimrod Glacier⁴¹, are widespread throughout the central Transantarctic Mountains and south Victoria Land. This intrusion of granites accompanied and followed a major tectonic movement, the Ross Orogeny, which was responsible for folding the Byrd Group and its probable correlatives.

Australia–Antarctica reassembly

Figure 1 shows comparative columns of the main elements of the stratigraphy and tectonics of the Cambrian sequences in Antarctica, South Australia, and Tasmania. A similarity is at once apparent between the northern Victoria Land (Bowers Group) and Tasmanian columns, constructed along the axial portions of both belts. The youngest fossiliferous marine deposits are in both cases overlain unconformably by dominantly continental coarse quartzose sandstones and conglomerates of probable latest Cambrian or earliest Ordovician age. They are both underlain by unfossiliferous clastic sequences with mafic volcanics; however, the volcanics form a major part of the Antarctic sequence but only a minor part of the Tasmanian succession. The Cambrian of both areas is intruded by Devonian–Carboniferous granites, in contrast to the other areas studied. Both successions were apparently deposited in rapidly subsiding trough-like structural depressions bounded by Precambrian blocks. Both regions were affected to a similar degree by tectonic activity particularly during latest Cambrian and Early Ordovician times, resulting in similar sequences of quartzose gravels and sands. It is likely, therefore, that western Tasmania lay very close to the Bowers Group depocentre in Cambrian and Early Ordovician times.

By contrast, fossiliferous Late Cambrian is absent from the southern South Australian sequences, while the Early and early

Middle Cambrian, which appears to be almost fully represented, contains prominent archaeocyathine limestone horizons. The contrast between the stratigraphy and geological history of the Cambrian of northern Victoria Land and that of South Australia shows that the sequences could never have been directly continuous, and the areas were unlikely to have been adjacent. However, a similarity is apparent between the archaeocyathine limestone-bearing Byrd Group of Antarctica and the South Australian sequences.

A reasonably firm geological basis for continental reconstruction between Australia and Antarctica has thus been established. The “geologic fit” proposed here (Fig. 1) differs markedly from the widely accepted “computer fit” based on morphology¹, but is relatively close to Griffiths’s “plate tectonic” reconstruction⁷. However, in this fit, Australia has been rotated anticlockwise slightly with respect to Antarctica so that the Cambrian of western Tasmania is nearly continuous with a possible northern extension of the Bowers Group. This reassembly has the added advantage of accommodating the South Tasman Rise with almost no overlap adjacent to the eastern coast of northern Victoria Land. The similarity of the quartzose semi-schist of the Rise with that of the Robertson Bay Group, and the coincidence of K/Ar age dates in both areas, has already been noted and correlation suggested by other workers⁴². It is interesting to note that the proposed fit, based on correlation of the Cambrian of western Tasmania and of northern Victoria Land, leaves the Archaeocyathine limestones of the Adelaide Geosyncline and of the Byrd Group in a position apparently far to the west of the projection of the Bowers Group–Dundas Trough alignment. This outcrop distribution could be accounted for by post-Cambrian strike-slip movement between Tasmania and mainland Australia causing segmentation of a once continuous but somewhat sinuous belt (as some writers^{43–46} have suggested), or pair of belts. An alternative possibility (rendered particularly attractive if Mawson’s reported discovery⁴⁰ of a possible Archaeocyathid in marble erratics at Cape Denison represents the northern extension of the Byrd Group) is that Cambrian sedimentation occurred in two distinct belts, the Early and Middle Cambrian Archaeocyathid-bearing one lying parallel to but well to the west of the Bowers Group–Dundas Trough depocentre. If the latter interpretation is valid, the necessity for major strike-slip movements between Tasmania and mainland Australia is eliminated.

The general similarity between the Cambrian fit proposed here and Palaeocene reassemblies of Antarctica and Australia based on “reversal” of seafloor spreading^{7, 8} is highly significant, implying that the relationship of the western Ross Sea sector of Antarctica to Tasmania and possibly to the whole of south-eastern Australia was substantially the same in the Cambrian and in the Palaeocene.

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Preliminary findings of the Viking gas exchange experiment and a model for Martian surface chemistry

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Oxygen and CO₂ were evolved from humidified Martian soil in the gas exchange experiment on Viking Lander 1. Small changes in N₂ gas were recorded. A model of the morphology and a hypothesis of the mechanistics of the Martian surface are proposed.

THE early results from the gas exchange experiment (GEX), on Viking Lander 1 have already been reported but were limited to preliminary findings only¹. This paper examines the findings and interprets them in terms of a model of the soil surface morphology and chemistry.

All major GEX activities that occurred after landing up to the end of the first incubation cycle are given in Table 1. In Table 2 are listed the sample processing and transport environments to which the soil was subjected up to the point when the

first analysis was made; they may have relevance to the extent and nature of the gaseous responses.

Data on instrument performance will be reported at a later date. Descriptions of the concepts governing the design of the experiment, modes of operation and results obtained are described elsewhere^{2–7}.

Humid mode results

The first incubation cycle began with the addition of 1 cm³ Martian soil to the 8.7 cm³ test cell (0.465 cm³ ± 0.035 cm³ solid volume, estimation based upon solid density range of 2.6–3.0 g cm⁻³ for the soil in the test cell and a bulk density of 1.3 g cm⁻³) (Viking Physical Properties Team). In the process of loading the soil and sealing the test cell, Martian atmosphere was trapped within the chamber at the prevailing pressure of 7.6 mbar (ref 8). A 0.132 cm³ volume of starting gas at 8.394 atm consisting of 5.51% Kr, 2.84% CO₂, and 91.65% He

Table 1 Major events in the first cycle of the gas exchange experiment

Event	Date (Sol)*	Elapsed time from landing (s)
Landing	0	0
Initiate chromatographic column rejuvenation	2	206,156
Soil sample added to test cell	8	682,426
Seal test cell	8	683,439
Add incubation gas, Kr/CO ₂ /He	9	759,219
Inject 0.57 ml nutrient (humid mode)	9	759,879
First headspace gas analysis (humid)	9	769,876
Second analysis	10	860,176
Third analysis	11	948,916
Fourth analysis	13	1,126,756
Fifth analysis	15	1,302,556
Inject 2.3 ml nutrient (wet mode)	16	1,383,339
First headspace gas analysis (wet)	16	1,392,916
Second analysis	17	1,481,656
Third analysis	18	1,570,456
Fourth analysis	20	1,744,456
Fifth analysis	25	2,189,320
Sixth analysis	28	2,446,105

The column rejuvenation cycle is the process by which the gas chromatograph columns, which have been opened to the vacuum of space, are cured of the need to be primed for oxygen in order to make quantitative measurements of oxygen in any given sample. The test cell had two positions on the carousel: the first to receive sample and the second to be sealed against the head-end. The latter contained all the attachments for adding gases and nutrient and for removing sample from the headspace. The soil was contained in a porous cup suspended over a sump that allows drainage, when required, and variable additions to either humidify or wet the soil. Test cell volume empty was 8.7 cm³ and the sample loop volume was 0.114 cm³. The amount of gas lost by sampling varied as a function of the total pressure in the test cell, the relative sample loop and headspace volumes and the gas distributions which were a function of temperature and gaseous species.

*Sol (the Martian day) is 24 h, 39 min, 35 s long.

Table 2 Soil sample history

Location	Temperature (K)	Time duration (h)
Soil surface (0–5 cm deep)	190–208	N.A.
Collection head (collect then dump)	204–206	0.09
Arrival at Biology PDA (soil processor) through shuttle (dump from PDA to SDA)	239–280	0.26
Arrival at Biology SDA (soil distribution) through soil dump to test cell	280–282	0.05
Arrival of soil in test cell through seal of test cell	282	0.28
Seal of test cell through humidification (addition of 0.57 ml nutrient)	282	21.2
Humidification through first analysis	282	2.78

Soil temperature estimated, all other temperatures directly measured.

and 0.567 cm³ of M4 nutrient³ were added to the lower cavity of the test cell, an amount insufficient to wet the soil but providing 100% relative humidity. The final pressure in the test cell after the above additions is estimated to be 150 mbar. Results of the first and subsequent gas chromatographic analyses of the headspace gases during the humid mode are shown in Table 3. After analyses of the headspace, the total gases in the test cell were estimated from Ostwald coefficients for aqueous solutions^{9–14} to determine the contribution of the gases dissolved in the liquid phase. The results were corrected for the initial contributions of the original trapped Martian atmosphere¹⁵, the starting Kr/CO₂/He gases and Ne introduced by the nutrient injection and losses from sampling the headspace gas. Solution coefficients were used for CO₂ to correct for the estimated basicity of the soil (Table 3). This technique was used only for CO₂ to approximate the magnitude of the CO₂ change.

The results show the CO₂, O₂, N₂ and Ar and/or CO (the Ar and CO are not resolved by this gas chromatograph) were evolved by the soil sample. The maximum amount of N₂, 83 nmol, appeared on Sol 11 (Sol is a Martian day, equal to 24 h, 39 min and 35 s) and decreased to 77 nmol by Sol 15. Oxygen, on the other hand, after reaching its maximum on Sol 11, appears to have reached a plateau. From the assumed total oxidation of ascorbate in the M4 nutrient³, the net amount of O₂ produced equals 775 nmol (690 plus the 85 associated with the oxidation of ascorbate). The maximum amount of CO₂ attained occurred on Sol 13 and was approximately, 9,800 nmol. This decreased on Sol 15 to 9,400 nanomoles. No conclusion on the presence or change of CO can be made because of the low values of the combined Ar/CO peak. The values in Tables 3 and 4 of Ne and Kr demonstrate the consistency of the internal standards and the apparent precision for the gas analyses.

Wet mode results

On Sol 16, additional (estimated volume = 2.268 cm³) M4 nutrient was injected. With the amount added earlier, the nutrient, now measuring 2.835 cm³, wetted the soil. The results for the wet mode are shown in Table 4. The level of N₂ monotonically rose for an increase of 11 nmol whereas in the humid mode we had seen an increase followed by a decrease in the N₂ content.

Oxygen monotonically decreased from the plateau that was attained at the end of the humid mode and reached a minimum. The total amount of O₂ consumed equalised 325 nmol which is 78% of the ascorbate-oxygen equivalence added.

With water now making contact with the soil, the gaseous content of the interstitial water could be estimated. For the 1 cm³ soil amount, an estimate of 5–50 µm particle sizes (personal communication from H. J. Moore, Viking Physical Properties Team) yielded an interstitial soil-water volume of about 35% (ref. 16). This value was used to estimate the total CO₂ in the solution contacting the soil, as separated from the CO₂ in the larger volume of water in the test cell sump that was treated separately as if it were unaffected by the soil. The interstitial soil solution and solution in the sump compartment shared a common headspace atmosphere from which the CO₂ was measured. The calculations show that CO₂ estimates are

only meaningful to the extent that they represent a minimum.

During the entire first cycle, no H₂, NO or CH₄ was observed in the headspace.

Model for Martian surface chemistry

From very preliminary laboratory data (personal communication from Dr Edward Merek, Ames Research Center) using terrestrial volcanic ash, it was observed that some of the CO₂ adsorbed at Martian surface temperatures, 190–268 K, was desorbed at the higher incubation temperature of the test cell,

Table 3 Gas composition (corrected) in gas exchange test cell (humid mode)

Gas	Time from humidification (h)				
	2.78	27.86	52.51	101.91	150.74
	Sol 9	Sol 10	Mars Date Sol 11	Sol 13	Sol 15
	Quantity of gas* (nmol)				
N ₂	73	76	83	79	77
O ₂	500	650	690	690	690
CO ₂	5,900	8,300	9,500	9,800	9,400
Ar†	8	7	13	9	8
Ne‡	20	19	18	20	21
Kr§	2,000	2,000	2,000	2,000	2,000

*The gas chromatograph detector data were sampled at 1 s intervals, digitised and fitted to a skewed gaussian distribution from which peak heights were obtained. The gas in the headspace was obtained from the ratio of the sample loop volume to the total headspace. The cumulative gas composition was corrected for sampling losses by referencing absolute changes in the krypton values for successive samples. Corrections were made for pressure sensitivity in this flight instrument caused by a partial restriction in the gas sampling system which prevented total evacuation of the sample loop to ambient pressure before filling (three times) from the test cell. The volume for krypton was corrected for pressure as follows:

$$\text{nmol Kr} = 37.77 (P_c) \exp -0.118 (V_p) \exp 1.016$$

where P_c is the test cell pressure in mbar, V_p is the peak height in volts. The value for each gas was corrected by the ratio of the term $37.77 (P_c) \exp -0.118$ to the similar Kr value from a pressure-insensitive instrument. P_c was determined from an inventory of all sources of pressure such as the enclosed atmosphere, the pressure of the initialisation gas injected and any additional injections of helium required to increase cell pressure. The gas composition as stated was corrected by removal of contributions from known sources (for example trace contaminants in injected gases) and for the amount dissolved in the liquid phase. An estimate of dissolved gases was made from literature values for the Ostwald coefficients. The effects of pH on the CO₂ distribution were included by estimating changes in apparent CO₂ levels upon nutrient injections in LR (second injection) and upon the wet-mode nutrient injection in gas exchange. The relationship used was

$$\frac{(\text{nanomoles, dissolved})}{(\text{nanomoles, gas phase})} = L \frac{(\text{volume, liquid})}{(\text{volume, gas})}$$

where the L values for CO₂ are as follows. Sol 9 and 10: $L = 21.4$; Sol 11–15: $L = 28.4$; Sol 16: $L = 40.4$; Sol 17–28: $L = 68.5$. The data shown in this table and in Table 4 are revised from those given in ref. 1 by better values for the atmospheric composition and the composition of the krypton/carbon dioxide gas. The correction, subtracting these contributions from the total, tend to increase the values, but do not alter the conclusions reached.

†Assumed to be argon (argon is not resolved from carbon monoxide on this column).

‡Mean value for neon = 20.02 ± 1.09 ($\pm 5.44\%$).

§Mean value for krypton = $1,975 \pm 0$ ($\pm 0\%$).

281–283 K, during the first 21.23 h that the soil was sealed in the test cell. However, when the same volcanic ash was then humidified, major desorption of the CO_2 occurred in 2.78 h. When the amount of CO_2 desorbed was related to the Martian soil, the 9,800 nmol of CO_2 reduced to 0.33 mg CO_2 desorbed per g soil. This value of desorbed CO_2 which is assumed to be adsorbed by Martian soil is within the model proposed by Fanale and Cannon¹⁷ and supports the method we used to estimate total CO_2 . The CO_2 production in the wet mode was probably due to oxidation of nutrient organic compounds.

Table 4 Gas composition (corrected) in gas exchange test cell (wet mode)

Gas	Time from 2.3 ml nutrient injection (h)					
	2.66	27.31	51.98	100.31	223.88	295.21
	Sol 16	Sol 17	Mars date Sol 18	Sol 20	Sol 25	Sol 28
Quantity of gas (nmol)						
N_2	76	78	78	79	86	87
O_2	670	600	550	510	460	450
CO_2	9,600	11,000	12,000	13,000	13,000	12,000
Ar	8	8	7	8	9	8
Ne	100	150	160	170	170	180
Kr	2,000	2,000	2,000	2,000	2,000	2,000

See Table 3 for details of test and calculations.

The unexpected amount of O_2 accompanying the desorption of CO_2 , 9,800 nmol/775 nmol (13 : 1 CO_2/O_2) from the minimum 270 : 1 CO_2/O_2 ratio in the Martian atmosphere¹⁵ represents an oxygen enrichment of approximately 20 times in the Martian soil. It is improbable that the excess amounts of O_2 found beyond that expected from adsorption of atmospheric O_2 comes from the direct or biological photocatalysis of water or hydroxyl groups, for the reasons that the critical temperature for O_2 is too low for adsorption in the quantities needed and the diffusion rate of O_2 at 7.6 mbar is too rapid to allow for enrichment to occur. A more likely source for the O_2 is chemically bound oxygen which is stable at GEX cell temperatures but allows displacement of oxygen by water. The surface of the grains of rock or mineral are good sources for this chemically bound oxygen. A simple estimate of the surface area of the grains can be made to assess whether all of the oxygen can be accommodated. The surface area of the grains are assumed to be 400–2,000 cm^2 per cm^3 of soil (personal communication from H. J. Moore for 5–50- μm particle sizes). Assuming 33.4 $\text{\AA}^2$ coverage per molecule, a monolayer of O_2 covers 1,560 cm^2 , and is accommodated by the available grain surface. The requirement that chemical absorption cannot extend beyond a monomolecular layer²⁶ is pertinent.

A model of surficial materials which can accommodate both oxygen and CO_2 is solid silicate particles covered with fine iron oxide particles. The carbon dioxide desorbed from a monolayer would be accommodated on 19,710 cm^2 of the iron oxide surface (assuming the 33.4 $\text{\AA}^2$ for CO_2 also). The iron oxide cover would be approximately three particles deep, under the conservative assumption that each iron oxide particle is a cube that has six times the surface area of the rock surface it covers. The least surface area is obviously a sphere having four cross sectional diameters of surface for each area of the mineral it would cover. If the mean particle size in the atmosphere (2 μm diameter) (ref. 19) is indicative of the iron oxide diameters on the surface, the mean thickness of the three-layer iron oxide is 6 μm . This is thick enough to make the inner surface opaque to the solar ultraviolet, but also too thick to be consistent with ≤ 2 μm thickness reported by the Inorganic Chemical Analysis Team (ICAT) on the limits of iron oxide coating allowed for measurement of underlying silicate surfaces¹⁸. If the value of the particle sizes were closer to the lower conservative estimate of 0.2 μm (ref. 20), the mean thickness would be compatible with the ICAT limit of detection.

These estimates of surface area are preliminary best estimates and may be revised at a future date. Lunar fines²⁵ have surface areas near 5×10^3 cm^2 g^{-1} while clays¹⁶ have surface areas in

the range of 10^5 – 10^7 cm^2 g^{-1} . The results of the GEX may ultimately yield an estimate of the surface area of the Martian surface materials.

The mechanical structuring of the oxygen affixed to the inner mineral surface instead of on the outer iron oxide coat is consistent with known chemicals, alkali and alkaline earth metal peroxides and superoxides that are capable of producing both O_2 and H_2O_2 in the presence of water vapour²¹. A candidate for one of the metal superoxides is calcium because of calcium's apparent abundance in the Martian soil¹⁸. For each superoxide hydrolysed by water, an equivalent alkaline site is produced that accounts for the resorption of the desorbed CO_2 . But in the natural state, the basicity of the inner surface is not transferred to the iron oxide coat, because the mineral surface is mechanically separated at the molecular level from the iron oxide coating of several layers. The H_2O_2 formed from superoxide hydrolysis will, in the presence of abundant iron oxide catalyst, directly participate in the oxidation of the exact number of oxidisable equivalents such as formate in the Labelled release (LR) nutrient²² or GEX M4 nutrient³. The alkali and alkaline earth metal superoxides are only one of many examples of the proposed chemistry on a stoichiometric basis that can well be applied to the ozonides, perchlorates, permanganates, and so on.

The iron oxide external surfaces provide at low temperature a monomolecular layer of adsorbed CO_2 which when activated by the solar ultraviolet flux splits off nascent oxygen and CO. The latter is free to return to the atmosphere. At higher temperatures, ultraviolet catalysis is limited by the quantity of CO_2 adsorbed. Other sources of nascent oxygen are photochemically formed ozone and hydrogen peroxide which are catalytically decomposed by the surficial iron oxide to form O_2 and O and H_2O and O , respectively. Then ascent oxygen, by simple diffusion (albeit an inefficient process, but time is not of the essence) passes through the porous iron-oxide matrix and attacks the inner mineral surface where it reacts with alkali and alkaline earth metal oxides to form the hydroxy-peroxides, superoxides, peroxyradicals and/or ozonides, if it does not first recombine with CO or react with another nascent oxygen. On the inner mineral surface, the products of the nascent oxygen reaction remain relatively stable but are potentially vigorous oxidisers.

If the oxygen–soil model is correct, it implies that the oxidiser source is distributed generally throughout the entire soil sample bed, that is, through the entire depth of the trench dug on the first sample taken. On the other hand, if the activity were associated only with the surface, there would have to be ten times the activity from just the surface material. If the red colour is associated with this activity, the colour photographic data substantiate that the entire trench is the same as the surface (personal communication from T. A. Mutch, Viking Lander Imaging Team). Furthermore, only a small fraction of the actual surface material is probably represented in the soil sample acquired. For example, a simple test of the surface sampler at JPL on surface tinted soil shows that only 11% of sieve size material 1.5 mm $> d > 0.589$ mm, 9% of 0.589 mm $> d > 0.495$ mm and 3% of 0.495 mm $> d$ are of surface origin in the test sample. A distribution of the oxidative capability throughout the entire depth of the trench, not just located on the exposed surface, indicates a history of stability consistent with the concept of the protective iron oxide coat under which is buried the oxygen-producing source. The stability is contrary to having the oxygen coupled surficially on the iron oxide coat because it would be at the mercy of transient changes in the vaporous environment over extremely long periods of time.

Another aspect of the iron oxide coating which bears on the stability question is related to the weak acidic nature of this material. Qualities which are ascribed to acid-like surfaces are the following: the ready emission of CO_2 from the soil in GEX, the small first peak from the pyrolytic release experiment (PR), the initial instant release of $^{14}\text{CO}_2$ from the LR and the

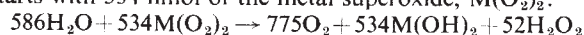
higher background counts when LR was cooled following heating of the test cell to sterilisation temperatures¹. The significance of the LR sterilisation regime is that the temperature is sufficient to volatilise such materials as $\text{H}_2\text{SO}_4 \cdot 2\text{H}_2\text{O}$ (boiling point 169°C) which can coat surfaces and prevent readsorption of $^{14}\text{CO}_2$ at the LR test cell temperature. The latter observation provided us with a basis for speculating that surficial acidity is provided by a thin coat of sulphuric acid from sulphate salts of weak bases (amorphous sulphate). A probable source of this sulphate is SO_3 produced by the visible activity of the past²³. The SO_3 reacts with any water vapour in the atmosphere to produce sulphuric acid that descends as a mist to cover the iron oxide coat. Such hygroscopic coatings could well preserve the integrity of the highly active inner superoxide coat during episodes of higher water vapour incidences on Mars while explaining durable O_2 production from the soil sample in the test cell. It may explain the observed cohesive nature of the Martian soil. The acid characteristic of the surface ascribed to amorphous sulphate is born out by the high level of sulphur measured by the ICAT (ref. 18) and the oxidised state of the surface as evidenced by the red colouration of the iron oxide outer coat. The acidic surficial characteristics are quickly neutralised by the strongly basic alkali and alkaline earth metal hydroxides formed by the hydrolytic reaction as demonstrated by both GEX and LR.

The above model of the silicate mineral covered with iron oxide requires that ultraviolet light initiate the process of nascent production where adsorbed CO_2 and/or H_2O is concentrated on the surface of the iron oxide particles. To maintain a steady-state concentration of these energy-rich oxygen bonds on the inner mineral surface, the combined dissipative effects of water vapour and heat must be counterbalanced by an equivalent influx of nascent oxygen. When temperatures are low and water vapour is virtually limited, as appears to be the case in the Lander 1 area, the oxygen sources are prevalent. Sites having more water vapour are expected to have less active material and produce less oxygen, but it remains to be determined whether the effective oxidative capacity, that is the capability to oxidise organics, is also reduced. The H_2O_2 product (unstable in the presence of iron oxide) may remain uncontacted by the iron-oxide outer coat.

The degree to which the particle surfaces are re-exposed to the ultraviolet flux to renew the activity, the penetration of nascent oxygen, hydrogen peroxide and/or ozone downward through the soil bed and the concentration of water vapour are factors that must be considered fully to describe and/or predict the potential to produce oxidisers at any site for any depth into the surface of Mars.

At the Viking Lander 1 site, the vista is an ancient one where the soil was formed before the boulders were strewn over it. This suggests that the soil was layered over fluvial scoured rocks well after the episodes of water formed the surface. The oxidisers could have been created by two distinctly different processes. During a period when there was a dense atmosphere, silicate particles could have been lifted into the atmosphere by winds allowing the surfaces to be exposed to solar ultraviolet thus creating oxidisers which have been preserved to this date. Oxidisers could also be formed constantly at a rate through the entire soil bed from atmospheric hydrogen peroxide, ozone or nascent oxygen providing a steady state production of oxidiser which counters the dissipative effects of warmth and humidity. Both ozone and hydrogen peroxide can provide nascent oxygen in our model.

The oxidisers in the Martian soil are good candidates for the reaction observed in the LR experiment¹ as well as some of the observed CO_2 evolution in GEX. They must have the requisite qualities of oxidising the formate in the LR, producing the O_2 in GEX and accounting for some of the CO_2 released in GEX. A reaction that could account for the above observations starts with 534 nmol of the metal superoxide, $\text{M}(\text{O}_2)_2$:



Thermodynamic calculations proving the validity of these

types of reactions in the environment simulating GEX will be tested at a later date.

The equivalent amount of oxidiser in the LR (26 nmol per 0.5 ml soil) calculated by one of us (V.I.O.), indicates that the inferred H_2O_2 produced as a result of soil reacting with water is limiting.

Because of the large abundance of water with respect to formate in the LR (ref. 22), 55.56 M per $2.4 \times 10^{-4} \text{ M} \sim 2.3 \times 10^5 \text{ l}^{-1}$, and in the GEX $1.9 \times 10^5 \text{ l}^{-1}$, it is unlikely that the superoxide will oxidise the formate directly, but will provide the requisite H_2O_2 in the reaction with water.

In the GEX, the N_2 changes are small and can be explained from either initial N_2 desorption from soil by water vapour and subsequent resorption in liquid water or biological fixation, while N_2 production could arise from a Van Slyke reaction where α -amino nitrogen in the M4 nutrient reacts on the aforementioned external-acid surfaces with nitrite in Martian soil. Since N_2 exists in the Martian atmosphere and there is postulated NO in the upper atmosphere²⁴, it would not be inconsistent to assume oxides of nitrogen exist in the Martian soil.

As we have observed from our studies of lunar soils^{6,7}, metallic iron formed by meteoric bombardment of lunar iron oxides provides a basis for estimating the biologically important, free-water history of a planet. At this time, no H_2 has been found, therefore no metallic iron can be inferred in the Martian sample tested by GEX. This suggests that an amount of water and/or O_2 necessary to oxidise the metallic iron to the depth of the regolith was available at some time for this oxidation. For answers on the question of the state of oxidation of the planetary surface as measured by the presence of metallic iron, we must await the recharge occurring at the start of the second cycle to remove O_2 so as to measure the production of H_2 from the oxidation of Fe^0 by H_2O , and await the findings of Lander 2. The ultimate sensitivity for H_2 detection will allow us to measure 0.003% of metallic iron in the Martian soil if O_2 is not present in the test cell.

The advantage of starting with metallic iron as a precursor source of iron oxide is that it presents iron in an easily oxidisable form that allows the propagation of the iron oxide product on a broad planetary scale without the need for liquid water. Only water vapour and O_2 are required. The early development of the iron oxide in the history of Mars explains how the dry lubricating qualities of the iron oxide dampens aeolian erosion and removes the need for liquid water to extrude iron oxide deposits from their sources.

We prepared earlier for an expected chemistry of an arid planet⁸ and must await the dissipation before life can be determined. At this time, all of the gas changes we have observed in our GEX test cell can be explained most easily by mechanistic processes. Although these results and explanations are preliminary, we find no need to invoke biological processes. While continued careful study is required, it is anticipated that results from Viking Lander 2 will reinforce our conclusions.

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Storage of elastic strain energy in muscle and other tissues

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Storage of strain energy in elastic materials has important roles in mammal running, insect jumping and insect flight. The elastic materials involved include muscle in every case, but only in insect flight is the proportion of the energy stored in the muscle substantial.

MUSCLE has elastic properties and so do the tendons and skeletal elements to which muscles attach. These properties are exploited by animals in two main ways. First, energy is saved, in insect flight and in mammal running, by storing elastic strain energy at one stage in the wingbeat or stride and releasing it at another^{1–3}. Second, some insects have catapult mechanisms which enable them to jump much higher than they otherwise could⁴. We have assessed the relative importance of the elastic properties of the muscles and of other structures in each of these activities. The arguments which follow show that the role of the elastic properties of the muscles is trivial in mammal running and insect jumping, but may be important in insect flight.

Capacity for elastic storage

Huxley and Simmons⁵ investigated the elastic properties of active frog semitendinosus muscle. They compensated for the compliance of the tendons by a spot-follower device so that they measured only the compliance of the muscle fibres themselves. Their observations indicated that elastic extension occurs almost exclusively in the crossbridges. Each active crossbridge is stretched by 15 nm. The experiments of Jewell and Wilkie⁶ with intact muscles indicated rather less stretching than this but those of Rack and Westbury⁷ indicated that the crossbridges were stretched by about 30 nm.

Consider a muscle fibre of length l , sarcomere length λ exerting a force P . Let each crossbridge suffer an elastic extension b so that each sarcomere extends $2b$. The fibre as a whole extends $2bl/\lambda$ and the elastic strain energy U is $P/2$ times this

$$U = Pbl/\lambda \quad (1)$$

Let the mass of the fibre be m and its density ρ so that its

volume is m/ρ and its cross-sectional area $m/\rho l$. The stress σ in the fibre is given by

$$\sigma = P\rho l/m \quad (2)$$

From equations (1) and (2) the strain energy per unit mass of muscle is

$$U/m = \sigma b/\lambda\rho \quad (3)$$

Let the maximum stress which can be developed in isometric contraction be σ_0 , and the corresponding strain energy U_0 .

We have calculated values of U_0/m for various muscles, assuming that active crossbridges stretch 15–30 nm in the invertebrate muscles as well as in vertebrate striated muscle (Table 1). The values of U_0/m are all fairly similar because σ_0 is likely to be roughly proportional to $\lambda^{1/3}$.

The maximum work which can be done by a muscle in a single contraction is the area under a graph of isometric force against length. From Fig. 1 this can be shown to be 220 J kg⁻¹ for frog striated muscle and 420 J kg⁻¹ for a crayfish muscle with longer sarcomeres. Notice that these values are of the order of 100 times greater than the capacity of muscle for storing elastic strain energy.

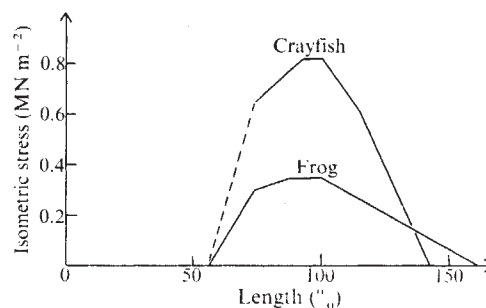


Fig. 1 Graphs of isometric stress against muscle fibre length for frog semitendinosus^{8,15} and crayfish extensor carpopoditi¹⁶. Length is expressed as a percentage of the length at which the isometric stress is maximal.

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Now consider a muscle in series with a tendon of length l_t . When it exerts a force P it stretches the tendon by Δl_t and the elastic strain energy stored in the tendon is

$$U_t = \frac{1}{2} P \Delta l_t \quad (4)$$

This can be expressed as a multiple of the elastic strain energy in the muscle, which is given by equation (1)

$$U_t/U = (\lambda/2b)(\Delta l_t/l) \quad (5)$$

$\lambda/2b$ is 35–70 for vertebrate striated muscle and rather more for some insect muscles (Table 1). Tendons stretch about 10% (ref. 14) and insect apodemes about 3% before breaking¹². Δl_t may thus be almost 0.1 l_t for tendons and 0.03 l_t for apodemes, if they are not much thicker than they have to be to withstand the forces their muscles exert on them. Hence U_t/U is likely to be greater than l_t/l . Pennate muscles with short fibres and long tendons or apodemes (that is, with large values of l_t/l) are likely to store far more elastic strain energy in their tendons or apodemes than in their muscle fibres.

Running

When a rubber ball bounces it loses kinetic energy and then regains most of it. The energy is stored briefly as elastic strain energy and converted back to kinetic energy in the rebound. Similarly a running mammal loses and regains kinetic and potential energy in every step. Some of this energy is stored as elastic strain energy in muscles and tendons and restored in an elastic recoil. The rest has to be replaced by metabolic work. Measurements of the oxygen consumption of running men and hopping kangaroos indicate that as much as 50% of the energy which would otherwise be needed for locomotion may be saved in each case by elastic storage^{2,3}.

Table 1 Strain energy stored in the series elastic component of various muscles in isometric contraction, calculated by equation (3) assuming a density of 1,060 kg m⁻³

Muscle	Vertebrate striated	Locust flight	Hemipteran flight	Locust leg
Sarcomere length, λ (μ m)	2.1	3.9	2.6	8.5
Maximum isometric stress, σ_0 (kN m ⁻²)	350	350?	120	800
Strain energy U_0/m (J kg ⁻¹) for:				
$b = 15$ nm	2.4	1.3	0.7	1.3
$b = 30$ nm	4.7	2.5	1.3	2.7
Reference	8	9	10, 11	12

The only leg muscles which can save energy in this way are the ones which first extend and then shorten while their foot is on the ground. The extensors of the ankle do this and so do the extensors of the knee (except in wallabies) but the other major leg muscles do not^{3,17}. The extensors of the ankle and knee are pennate with long tendons, the Achilles and patellar tendons. Table 2 shows that far less elastic strain energy is stored in muscle fibres than in the Achilles tendon, in running men, hopping wallabies and jumping dogs. Strain energy has been calculated for the ankle and knee extensor muscles together, but for the Achilles tendon alone. We have not enough data to make

reliable estimates of the elastic strain energy stored in the patellar tendon, but it is plainly substantial. The discrepancy between the quantities of strain energy stored in the muscles and tendons must be even more marked than is apparent from Table 2.

The data for man is less reliable than the other data in Table 2. The value for muscle mass m was obtained from a dissecting-room cadaver (albeit an unusually robust one) and is almost certainly less than the mass of the corresponding muscles in the men who made the force-platform records. If so, the stress σ in the muscles will have been overestimated so the value of U (which is proportional to $m\sigma$, equation (3)) is probably about right.

Insects jumping

A boy using a catapult does work relatively slowly on the rubber, storing elastic strain energy which is released very rapidly in the elastic recoil. The power output of the catapult is thus much greater than the power input: the catapult serves as a power amplifier. Elastic structures are used in the same way by jumping insects. They are needed for the following reason.

Suppose an animal has muscles which, like vertebrate striated muscle, can do work amounting to 220 J kg⁻¹ in a single contraction. Suppose that the jumping muscles make up 5% of the mass of the body. They are in principle capable of giving the body 11 J kinetic energy per kg body mass, that is of accelerating it to a take-off velocity of 4.7 m s⁻¹ which is enough for a vertical jump in which the centre of mass rises 1.1 m. This is true, whatever the size of the animal. However, a small animal must accelerate to its take-off velocity over a very short distance, in a standing jump: it must give itself the kinetic energy in a very short time so the power output required (per unit mass of muscle) is high. Locusts take off at 3.2 m s⁻¹ or less and the jumping muscles make up 7.5% of body mass so the work the muscles have to do is only 70 J kg⁻¹. However, the power

required is 5,000 W per kg muscle, about ten times the maximum power output of the fastest known muscle¹⁹. Locusts and other jumping insects can only jump as well as they do because they have catapult mechanisms. Their leg muscles contract, storing elastic strain energy in themselves, their apodemes and other structures. This energy is released very rapidly at take-off, by a trigger mechanism.

Nearly all the energy for a locust jump is supplied by the extensor tibiae muscles. Table 1 shows that the elastic strain energy stored in these muscles before a jump is unlikely to be more than 2.5 J kg⁻¹, a very small fraction of the 70 J kg⁻¹

Table 2 Calculations of elastic strain energy in the muscles and in the Achilles tendon of one leg of a man running at 3.9 m s⁻¹, a wallaby (*Protemnodon rufogrisea*) hopping at 2.4 m s⁻¹ and a dog taking off for a running long jump

	Man	Wallaby	Dog
Mass of animal (kg)	68	10.5	26
Mass of muscles, m (kg)	1.66	0.48	0.37
Muscle strain energy, U (J)	4.1–8.3	0.36–0.73	0.6–1.1
Force in tendon, P (N)	4,700	850	2,000
Extension of tendon, Δl_t (mm)	18	11	25
Tendon strain energy, U_t (J)	42	4.7	25
Reference	18	3	14

The muscles referred to are the extensors of the ankle and knee. The strain energy in them has been calculated from equation (3), using the stresses calculated in the references and the values of b and λ given in Table 1. The extensions of Achilles tendon in the wallaby and dog have been taken from references 3 and 14; the value for man has been calculated in the same way, assuming a cross-sectional area of 70 mm². The strain energy in the tendon has been calculated by equation (4).

which is needed. The rest is apparently stored as elastic strain energy in the apodemes of the muscles and in the semilunar processes of the knee joint¹².

Similar conclusions can be drawn from our knowledge of jumping by fleas and click beetles. The kinetic energy at take-off is around 20 J per kg muscle and 60–100 J per kg muscle for strong jumps by the flea *Spilopsyllus* and the beetle *Athous*, respectively^{20,21}. Only a little of this can be stored as elastic strain energy in the muscles. Most of it is stored in other structures, in fleas mainly in pads of the elastic protein resilin.

Table 3 Assessment of the contribution of elastic strain energy in muscles to jumping by locusts and fleas

	Locust	Flea
Length of muscle fibre (mm)	4	0.3
Fractional shortening required, ϵ	0.23	0.11–0.20
Sarcomere length (μm)	8.5	5
Expected elastic strain, ϵ	0.007	0.012
Muscle strain energy, ϵ		
Total work	0.03	0.06–0.11
Reference	12	20,32

The geometry of the legs requires the muscle fibres to shorten by the fraction ϵ , to extend the legs at take off. If each crossbridge stretches by 30 nm the elastic strain of the muscle fibres will be ϵ' .

Table 3 confirms the conclusions of the past few paragraphs. It shows by a different argument that elastic strain energy in the muscles can contribute very little to jumping by locusts and fleas.

Insect flight

The role of elastic storage in insect flight is very like its role in running. Kinetic energy taken from the wings at the end of one stroke is stored as elastic strain energy and used in the next stroke. As much as 50% of the power which would otherwise be needed for flight may be saved by elastic storage^{1,22}.

The flight muscles of locusts in level flight deliver 170 W kg^{-1} (ref. 23). The frequency of the wing beat is 19 Hz so the work done by the muscles in each contraction is 9 J kg^{-1} . As much as 2.5 J kg^{-1} may be stored as elastic strain energy in their fibres (Table 1) depending on the stress which is developed. Larger quantities of elastic strain energy are stored in cuticular structures (mainly in structures made of resilin, ref. 24) but the contribution of muscle elasticity cannot be neglected. Many insects beat their wings at much higher frequencies but their muscles deliver little or no more power²² so the work per cycle is much less and the possible contribution of muscle elasticity correspondingly more important. Many of them have myogenic muscles which shorten between 1% (in *Bombus*) and 3% (in *Calliphora*)²⁵. In such muscles, the sarcomeres are around $2.5 \mu\text{m}$ long, so if the crosslinks can strain elastically by 30 nm, elastic strains of 2.2% are possible.

In oscillatory contraction these muscles exert greater forces during the shortening half cycle than during the lengthening half cycle. A graph of force against length (or of moment against wing angle, Fig. 2a) is a loop of which the area represents the work done in each cycle. The gradient of the long axis of the loop is the elastic stiffness of the muscle and approximately corresponds to the graph of force against length for the active muscle in the non-oscillatory condition.

The power output of such muscles is high. The output of flight muscles of the beetle *Oryctes* is 30 W kg^{-1} (at about 40 Hz) and for *Bombus* muscle (at a higher frequency) 60 W kg^{-1} , both measured *in vitro*²⁶. Estimates based on the metabolic rate and the probable efficiency of muscle suggest that bees and flies may be able to produce muscle power outputs in the order of 200 W kg^{-1} (calculated from data of Weis-Fogh, in ref. 26). As the muscle seems to be able to store up to 1.3 J kg^{-1} (Table 1), it seems that at the quite moderate frequency—for a small insect with myogenic muscles—of 100 Hz, the muscle could store and release 130 W kg^{-1} which is of the same order as the active power output. The muscles should thus act as major

stores to conserve the kinetic energy of the wings and as such should bring about harmonic oscillation of the wings.

It is unfortunate for this argument that simple harmonic oscillation of the wings rarely occurs (see ref. 27). It is usual to find that the angular velocity of the wing is fairly constant during both up and down strokes and that the wing direction changes abruptly at the extremes of the stroke^{28,29}. This cannot be explained by the simple harmonic motion of an elastic oscillating flight motor.

One mechanism which causes this is the dipteran click mechanism³⁰. The click mechanism in isolation acts in a manner similar to an electric light switch; at the two extremes of the travel, the mechanism is stable and the wing sets either top dead centre or bottom dead centre. When moved but a short distance from the extreme position, the wing when released snaps back to the extreme position but when moved beyond its mid-point, the wing continues to move until it clicks into the other extreme position. Depending on the geometry of the system, the mechanism can have a greater or lesser effect on the wing movement.

Figure 2c is a schematic graph showing the moment exerted by the click mechanism at different wing angles. Muscle elasticity tends to restore the wings to their mid position but the click mechanism acts in opposition to it, tending to send the wings to one or other extreme position. The click mechanism does not absorb energy (Fig. 2c shows a line, not a loop) but in the middle part of the stroke it stores elastic energy while the muscles are releasing elastic energy and vice versa. Figure 2d shows how the forces exerted by the click mechanism probably affect the other forces which act on the wing. They reduce or eliminate the inertia force in the middle section of the stroke so the wing accelerates early in the stroke, moves at fairly constant velocity in the middle part of the stroke and decelerates again at the end.

The click mechanism has other functions. It brings about the rapid torsional movements of pronation at the start of the downstroke and of supination at the bottom of the downstroke^{27,30}, which are utilised in the clap-fling and flip mechanisms of lift generation proposed by Weis-Fogh²². The energy

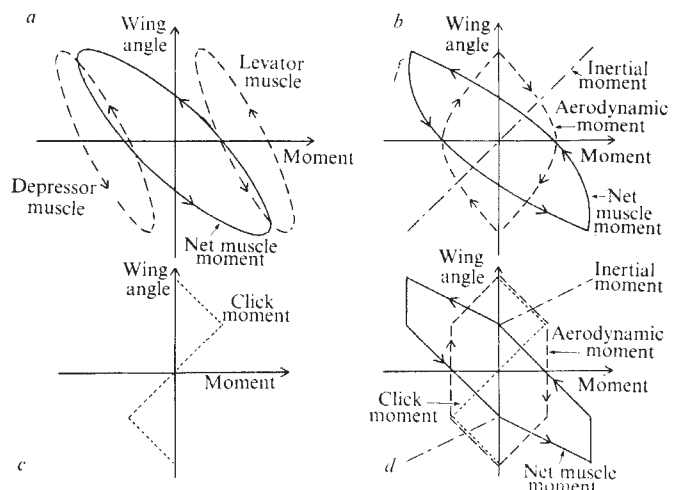


Fig. 2 Schematic graphs showing moments which act on an insect wing throughout the wing beat. Wing angle is regarded as positive when the wing is elevated. Moments are regarded as positive when they act dorsalwards. *a*, Moments exerted by muscles. The moments exerted by the levator and depressor muscles can be added together to give the net muscle moment. *b*, Moments on a wing which has no click mechanism. The moments shown must total zero at all stages in the wingbeat cycle. Inertial moment is shown proportional to wing angle, implying simple harmonic motion. *c*, Moments exerted by the dipteran click mechanism. *d*, Moments on a wing with a click mechanism. The moments shown must total zero at all stages in the wing beat. Readers will observe that the schematic graphs of net muscle moment in *a*, *b*, and *d* differ a little in shape. We do not know the precise shape of the loop for any intact insect and have obtained the shapes shown by making simple assumptions about the shapes of the graphs of other moments.

for these rapid movements is derived from the energy produced by the flight motor and the resultant kinetic energy of rotation of the wings, which is absorbed at the extremes of the wing stroke by the click mechanism and returned as rapid angular acceleration and torsional rotation of the wing. Thus the click mechanism acts as both a skeletal energy store and a power amplifier. Furthermore, since conventional cuticle can store $0.5\text{--}2\text{ kJ kg}^{-1}$ (ref. 31), the mass of the click mechanism may be as little as 1% of that of the flight muscles.

Role of series elastic component in locomotion

The series elastic component of muscle operates over small strains. Hence it can only store a small part of the work done by a muscle which shortens by a large fraction of its length. Also, it can only be responsible for a small part of the total store of elastic strain energy if the muscle is in series with a long compliant tendon. For these reasons it is relatively unimportant in insect jumping and mammal running. However, it is probably important in myogenic insect flight muscles, which operate over very small strains.

The quantity of elastic strain energy which can be stored in unit mass of material is 5 J kg^{-1} or less for muscle (Table 1) but it is $2,000\text{--}9,000\text{ J kg}^{-1}$ for tendon collagen, insect apodeme and resilin⁴. A structure for storing elastic strain energy can be much smaller if it is made of one of these latter materials than if it is made of muscle. The amount of work which can be done by a muscle in a single contraction may be 400 J kg^{-1} or more (Fig. 1) so a muscle designed to do a given amount of work will be far too small to store a similar amount of elastic strain energy. Hence the capacity of muscles for storing elastic strain energy is generally too small to be useful.

Insect flight muscles operate over very small strains so they do very little work in each contraction. However, they could not be replaced by smaller muscles working over larger strains because the power output required of them (around 200 W kg^{-1}) is apparently close to the maximum available from muscle¹⁹. Their capacity for elastic storage is large enough to be extremely useful. In general, the elastic strain energy to be stored in each cycle of wing movement is similar in quantity to the net work done by the muscles²². If the wing frequency is 20 Hz and the power output of the muscles 200 W kg^{-1} , the work done in each cycle is 10 J kg^{-1} and the capacity of the muscles for storing elastic strain energy may need supplementation. If the frequency is 200 Hz the work done per cycle (for the same power output)

is only 1 J kg^{-1} and the capacity for storing strain energy may be ample even when partially counteracted by a click mechanism. Large insects such as locusts fly with low wingbeat frequencies and have structures such as relatively large resilin pads to supplement the elastic storage capacity of their muscles. Dipteran flies, being smaller, have higher wingbeat frequencies, and use click mechanisms which partly counteract the elastic compliance of the muscles. Their resilin hinges are relatively small.

Storage of elastic strain energy in muscles (or in tendons or apodemes in series with muscles) must imply an energy cost, since energy is needed to develop and maintain tension in muscle. We are unable to assess this cost for any of the activities we have discussed, but note that it will be least with slow muscles where storage is brief relative to the cycling time of the crossbridges.

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DNA base sequence of the p_o promoter region of phage λ

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The sequence of a 123 base pair HpaII restriction fragment of bacteriophage λ DNA has been determined by the dimethylsulphate-hydrazine technique. Of this fragment 65 nucleotide pairs are transcribed into the 5' proximal part of the oop RNA. The remaining 58 nucleotide pairs preceding the start point of transcription show homologies to the λp_L and λp_R promoter regions, and are concluded to contain the λp_O promoter sequence.

REPLICATION of bacteriophage λ DNA proceeds bidirectionally¹ starting from a well defined region positioned between 79.9 and 80.6% on the λ physical map². All known *cis*-dominant mutants affecting the origin of replication (*ori*) map within this

region^{3–6}. A small RNA molecule of known sequence⁷ is transcribed from the *I* strand of this DNA segment (79.9–81.1) (ref. 8), and there is evidence that this RNA acts as a primer in the initiation of λ DNA replication⁹. Both the synthesis of this *oop* RNA, and the initiation of λ DNA replication require the products of the phage genes *O* and *P* as well as the *dnaB* and the *dnaG* gene products of the host^{9,10}. In addition, mRNA synthesis initiated in this area has been shown to be involved in transcription leading to repressor establishment during lysogenisation¹¹. This suggests that the *oop* RNA may be equivalent to a mRNA leader transcript. Because of this role in lysogenisation, phage gene *cII* and *cIII* products may also modify initiation or attenuation¹² rates of the *oop* RNA synthesis.

As a step towards elucidating the structural features of this

area in more detail we have undertaken to sequence the promoter region of the *oop* RNA. The 81-nucleotide sequence of the *oop* RNA as reported in ref. 7 contains a CCGG sequence at positions 68–71, which indicates a restriction enzyme *HpaII* sensitive site¹³ for the corresponding DNA sequence. Therefore, the generation of a restriction fragment of at least 70 base pairs could be expected with this enzyme. This fragment would represent the DNA complement of most of the *oop* RNA sequence and perhaps part or all of the *p_o* promoter region. In order to deal with a smaller number of *HpaII* restriction fragments, we have used the small λ -derived plasmid λ dvh 93 (ref. 14) instead of DNA from the whole λ phage (see Fig. 1). Cleavage of λ dvh 93 DNA with *HpaII* enzyme produces 10 major fragments, 7 of which have more than 70 base pairs (A to G, Fig. 1). For further selection among those *HpaII* fragments, the fragment λ dvh 93–*HindII*·C (ref. 14) was isolated, since it spans the region where the *oop* RNA has been mapped (Fig. 1). Only four of the fragments with more than 70 base pairs were produced on digestion with enzyme *HpaII*: fragments B, C, E and F (Fig. 1). Fragment T, which is not observed in the *HpaII* digest of total λ dvh DNA, comprises the terminal portion of the *HindII*·C fragment that contains the terminator region of the *oop* RNA (E. Schwarz, unpublished work from this laboratory, and ref. 15). The decanucleotide d(T-G-G-A-T-C-T-A-T-C) whose sequence is complementary to the nucleotides 4–13 of the *oop* RNA sequence (see Fig. 4) had been synthesised chemically in our laboratory (D. Ghosal, unpublished). In a reaction catalysed by DNA polymerase I with linearised and denatured λ dvh 93 DNA as a template, this decanucleotide acts as a primer near the *p_o* promoter region. When the isolated and denatured *HpaII* fragments B, C, E and F were tested in four parallel experiments

as templates in such reactions, only the fragment *HpaII* F gave primer-dependent products (D. Ghosal, in preparation).

Sequence determination of the fragment *HpaII*·F

For determination of the DNA sequence of the restriction fragment by use of the dimethylsulphate/hydrazine technique developed by W. Gilbert and A. Maxam, the fragment *HpaII* F was first labelled at its 5' ends using polynucleotide kinase and [λ -³²P]-ATP. As no internal restriction enzyme sensitive site was available for separating the two labelled 5' ends, strand separation by electrophoresis on a 10% polyacrylamide gel¹⁶ had to be performed (Fig. 2). In order to reduce cross contamination of the two strands, both were purified further by a second strand separation cycle. The dimethylsulphate-hydrazine technique (W. Gilbert and A. Maxam, personal communication, and ref. 17) consists of four parallel reactions, two of them being purine specific, with dimethylsulphate as a reagent, the other two being pyrimidine specific, using hydrazine. The products of the four limited (one random hit per chain) degradation reactions are fractionated according to chain lengths in adjacent slots on a denaturing polyacrylamide gel. The patterns of radioactive products are then made visible by autoradiography. The methylation conditions for the G and A specific reactions are identical, but the release of the methylated bases is different: neutral release at higher temperature (15 min at 90 °C) is more specific for the methylated G sites and results in a final gel pattern with heavy bands representing polynucleotides terminating at the G positions; only light bands are obtained from the A positions ('G-specific' reactions). Acid release (60 min at 0 °C in 0.1 N HCl) gives the opposite result, dark bands for every A position and light bands for every G position

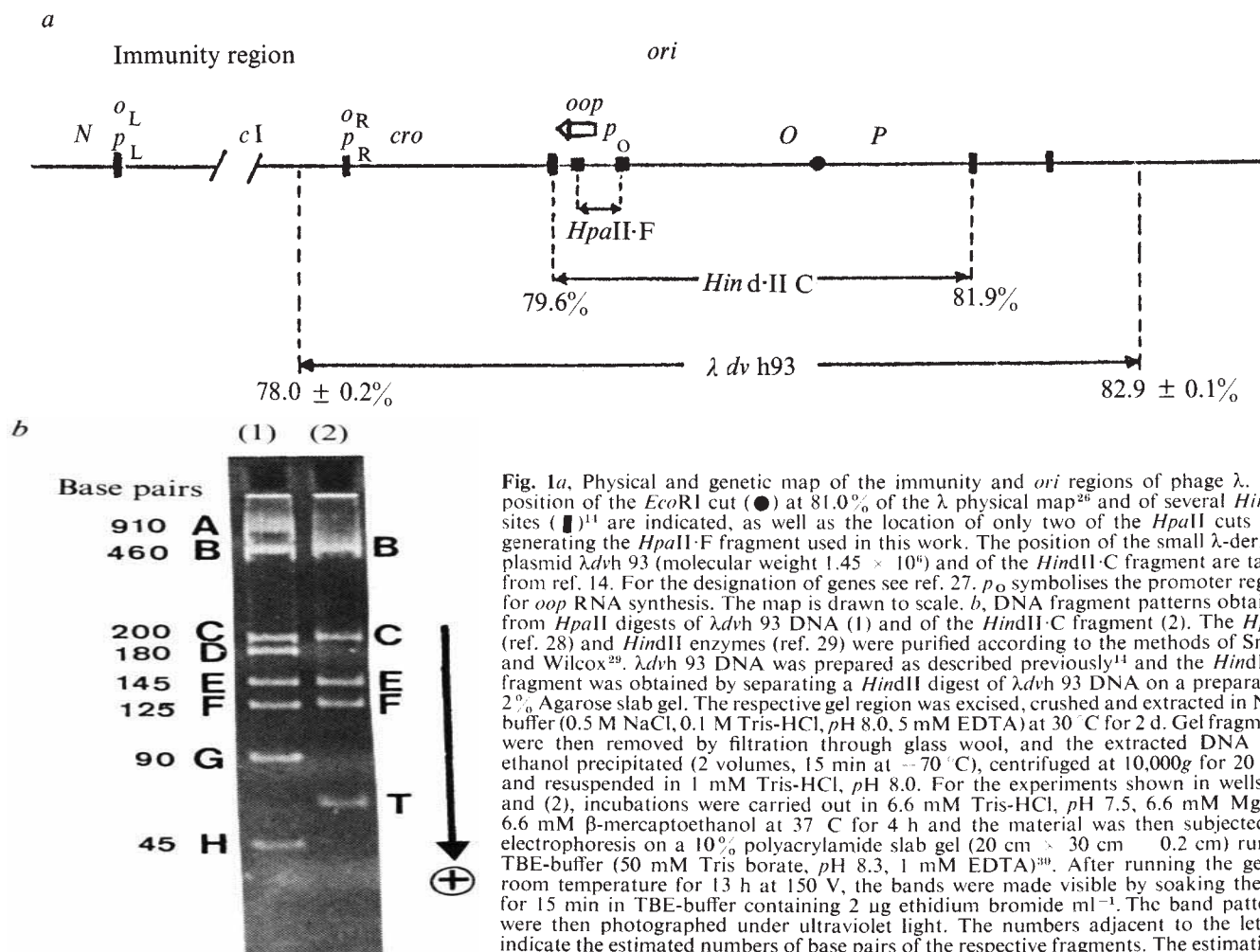


Fig. 1a, Physical and genetic map of the immunity and *ori* regions of phage λ . The position of the *EcoRI* cut (●) at 81.0% of the λ physical map²⁶ and of several *HindII* sites (■)¹⁴ are indicated, as well as the location of only two of the *HpaII* cuts (■) generating the *HpaII*·F fragment used in this work. The position of the small λ -derived plasmid λ dvh 93 (molecular weight 1.45×10^6) and of the *HindII*·C fragment are taken from ref. 14. For the designation of genes see ref. 27. *p_o* symbolises the promoter region for *oop* RNA synthesis. The map is drawn to scale. b, DNA fragment patterns obtained from *HpaII* digests of λ dvh 93 DNA (1) and of the *HindII*·C fragment (2). The *HpaII* (ref. 28) and *HindII* enzymes (ref. 29) were purified according to the methods of Smith and Wilcox²⁹. λ dvh 93 DNA was prepared as described previously¹⁴ and the *HindII*·C fragment was obtained by separating a *HindII* digest of λ dvh 93 DNA on a preparative 2% Agarose slab gel. The respective gel region was excised, crushed and extracted in NTE buffer (0.5 M NaCl, 0.1 M Tris-HCl, pH 8.0, 5 mM EDTA) at 30 °C for 2 d. Gel fragments were then removed by filtration through glass wool, and the extracted DNA was ethanol precipitated (2 volumes, 15 min at –70 °C), centrifuged at 10,000g for 20 min and resuspended in 1 mM Tris-HCl, pH 8.0. For the experiments shown in wells (1) and (2), incubations were carried out in 6.6 mM Tris-HCl, pH 7.5, 6.6 mM MgCl₂, 6.6 mM β -mercaptoethanol at 37 °C for 4 h and the material was then subjected to electrophoresis on a 10% polyacrylamide slab gel (20 cm $\times$ 30 cm $\times$ 0.2 cm) run in TBE-buffer (50 mM Tris borate, pH 8.3, 1 mM EDTA)³⁰. After running the gel at room temperature for 13 h at 150 V, the bands were made visible by soaking the gel for 15 min in TBE-buffer containing 2 μ g ethidium bromide ml^{–1}. The band patterns were then photographed under ultraviolet light. The numbers adjacent to the letters indicate the estimated numbers of base pairs of the respective fragments. The estimations were done by comparison with known restriction fragments of λ dv DNA (ref. 14).

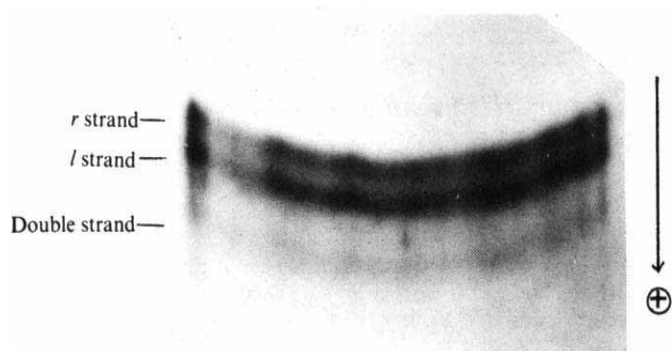


Fig. 2 DNA strand separation of fragment *HpaII*-F. Preparative amounts of the restriction fragment *HpaII*-F were obtained by cleaving 300 μ g of λ dvh 93 DNA with enzyme *HpaII*, separating the products on a preparative 10% polyacrylamide slab gel (20 cm $\times$ 30 cm $\times$ 0.4 cm) and extracting the fragment as described in Fig. 1. For the 5'-terminal labelling, about 10 pmol of fragment *HpaII*-F were incubated with 0.2 units of alkaline phosphatase from calf intestine (grade I, Boehringer-Mannheim Biochemicals) in 10 mM Tris-HCl, pH 9.3 at 37 °C in a total volume of 40 μ l. The reaction was stopped after 30 min by the addition of 8 μ l of 50 mM EGTA and boiling the whole mixture in a sealed

capillary for 3 min. 200 pmol of [γ^{32} -P]-ATP (prepared by the method given in ref. 31 with a specific activity of about 1,000 Ci mmol $^{-1}$) and 5–8 units of polynucleotide kinase (Boehringer-Mannheim Biochemicals) were added with 8 μ l 10 $\times$ kinase buffer (0.4 M Tris-HCl, pH 9.3, 0.1 M MgCl $_2$, 10 mM DTE, 50% glycerol) and the reaction mixture (80 μ l) was incubated for 30 min at 37 °C. After terminating the reaction with 10 μ l of 0.5 M EDTA, the mixture was adjusted to 0.3 M sodium acetate. Ethanol precipitation was then carried out in the presence of 100 μ g of carrier tRNA (2 volumes ethanol, 15 min at –70 °C). The dried pellet was resuspended in 300 μ l of strand separation solution (10% glycerol, 0.3 N NaOH, 1 mM EDTA) according to W. Gilbert and A. Maxam (personal communication) and loaded on a 10% polyacrylamide slab gel in a sample well of 8.5 cm $\times$ 0.4 cm. The gel was run in TBE-buffer at 4 °C and 300 V, until the xylene cyanol FF marker applied in a separate well had reached the bottom of the gel (30 cm). X-ray film (Agfa Gevaert Osray T4) was exposed for 30 min to the gel covered with polyethylene wrap. Under the conditions given, the *r* and *l* strands (at 20.0 and 20.8 cm from the origin) were clearly separated. The small band seen below the separated strands represents undenatured (or renatured) double-stranded DNA. The regions containing the separated strands were cut out and extracted twice with 2 ml each of NTE buffer at 30 °C for two 24 h periods; this led to about 90% recovery of radioactivity. On running a second strand separation cycle (not shown) the final yields ranged between 1×10^5 – 2×10^5 c.p.m. per strand, as measured by Cerenkov radiation.

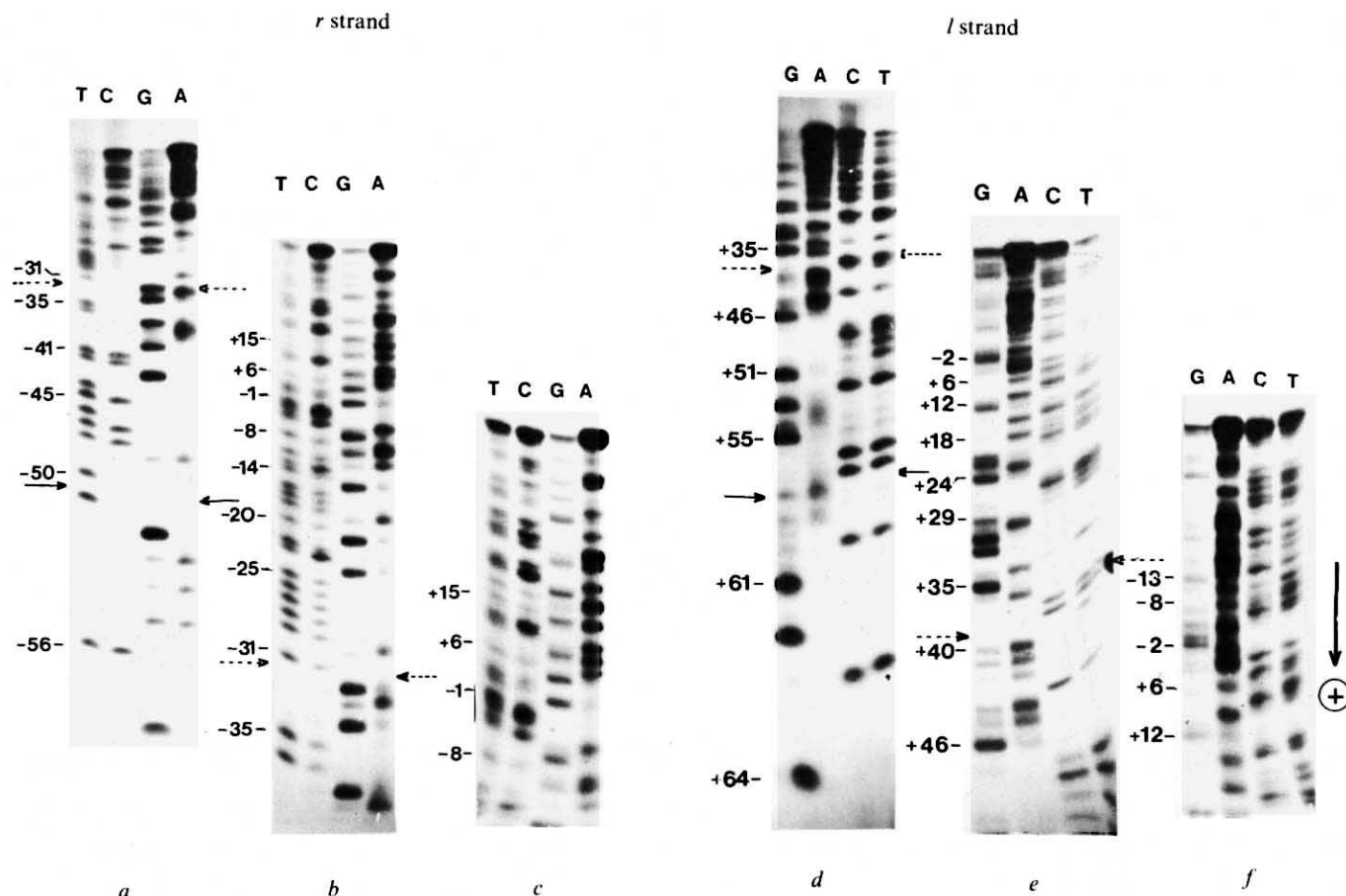


Fig. 3 Determination of the sequence of the *r* strand and the *l* strand of fragment *HpaII*-F with the dimethylsulphate-hydrazine technique. 80 μ g of sonicated calf thymus DNA were added to the separated strands, isolated as described in Fig. 2. After ethanol precipitation the material (about 1×10^5 Cerenkov counts) was divided into four equal parts. The two dimethylsulphate reactions (G and A specific; 30 °C and 37 °C respectively) were carried out in 500 μ l of 50 mM sodium cacodylate, pH 8.0, 10 mM MgCl $_2$, 0.1 mM EDTA containing 1–2 μ l of 10.7 M dimethylsulphate; the two hydrazine reactions were carried out at 37 °C in volumes of 50 μ l (15 M hydrazine for the T-specific and 15 M hydrazine, 1 M NaCl for the C-specific reaction). The work-up of the reaction mixtures followed essentially the protocol of W. Gilbert and A. Maxam (personal communication and ref. 17). Incubation times were 60 min for the experiments shown in (a) and (d), 45 min for (b) and (e) and 30 min for (c) and (f). The cleavage products were finally fractionated on a 20% denaturing polyacrylamide slab gel (20 cm $\times$ 40 cm $\times$ 0.2 cm) containing TBE-buffer and 7 M urea. Electrophoresis was performed at room temperature applying 450 V for 17 h (a) and (d), 500 V for 42 h (b), 600 V for 21 h (e) and 600 V for 61 h (c) and (f). For the experiments shown in (a) and (d), a prerun was made at 600 V for 4 h. After electrophoresis the wet gels were covered with polyethylene wrap and exposed to X-ray film at –20 °C for 6–10 d. The numbers besides the autoradiograms refer to the corresponding positions of the *HpaII*-F sequences shown in Fig. 4. The solid and dashed arrows give the position of the bromophenol blue and of the xylene cyanol FF markers, respectively. The columns labelled, T, C, G and A show the products of the T-, C-, G- and A-specific reactions, respectively. The heavy bands on top of each column represent unreacted starting material.

('A-specific' reaction). Limited reaction with hydrazine and no salt leads to a final product pattern with equal intensities for every T and C position ('T-specific' reaction), while the addition of 1 M sodium chloride to the hydrazine reaction mixture results in dark bands for the C positions and in light bands for the T positions ('C-specific' reaction).

Figure 3 shows the product patterns obtained by this technique for the separated *r* and *l* strands of the DNA fragment *Hpa*II·F. Three autoradiograms taken from three different electrophoresis gels are shown for each strand. They add up to more than 70 nucleotides for each strand. The final sequence as derived from both complementary strands is given in Fig. 4.

The following comments about the autoradiograms should be noted.

(1) The enzyme *Hpa*II recognises the double-stranded equivalent of the sequence 5-CCGG-3 cutting after the first C to produce restriction fragments starting with CGG at the 5 ends¹³. However, the very first nucleotides of the chains could not be resolved, and the lowermost bands clearly visible in Fig. 3a and d indeed correspond to the third nucleotides from the 5 ends (nucleotide numbers -57 and +64, respectively). This can be deduced from the bromphenol blue marker, which indicates the position of fragments 10 nucleotides long and by comparing the sequence derived from the *l* strand with the overlapping portion of the DNA sequence reported in ref. 15.

(2) There are two positions in the autoradiograms shown, where the G-specific reaction gives dubious results: the G at position 11 (*l* strand) and the A at position -10 (*r* strand). In the first case, the G-specific reaction gives a rather weak band compared with the G band corresponding to position

12, while in the second case a rather strong band appears at an A-position (but in the G-specific reaction). These irregularities have proved to be reproducible in several experiments. The ambiguities could be resolved, however, by looking at the corresponding positions of the complementary strand sequence. Further independent proof for the GG sequence in position +11/+12 (*r* strand) comes from the fact that the synthetic decanucleotide d(T-G-G-A-T-C-T-A-T-C) containing this GG doublet can act as primer in this region (position +13 to +4) (D. Ghosal, unpublished work from this laboratory).

(3) The CTCCT sequence at positions -5 to -1 in the *r* strand is not clearly resolved, but by inspecting the corresponding product pattern derived from the *l* strand, the sequence AGGAG (-1 to 5) can clearly be seen.

(4) Occasionally 'ghost' bands can be detected; these are due to small cross contaminations by the complementary strand. They can easily be assigned to the complementary strand, if the products of the four reactions of both strands are fractionated side by side on the same gel. As an example, the three ghost bands at positions -48, -46, and -44 in the G-specific reaction of the *r* strand (Fig. 3a) represent the G positions +55, +53, and +51 of the contaminating *l* strand (Fig. 3d).

Sequence features of the *oop* DNA and of the *p_O* promoter region

The sequence of the fragment *Hpa*II·F as resulting from the autoradiograms shown in Fig. 3 is presented in Fig. 4 in two halves; the upper part shows the section (*oop* DNA) transcribed into *oop* RNA; the lower part represents the section not being transcribed including the *p_O* promoter region. For comparison, the sequence of the λ promoters *p_R* and *p_L* as taken from ref. 18 are included in this figure. The segment including positions +53 to +66 is identical to the corresponding part of a sequence reported in ref. 15. (From this overlap the position of fragment *Hpa*II·F on the physical map of λ , as shown in Fig. 1, could be deduced.) From the DNA sequence determined we predict a sequence for the *oop* RNA (written as the top line in Fig. 4), which deviates from the reported sequence⁷ at three positions (underlined in Fig. 4) as follows.

(1) According to the present data the triplet GCC has to be inserted at the positions 60 to 62, which is in accordance with this part of the sequence given in ref. 15. (2) At position 46 a

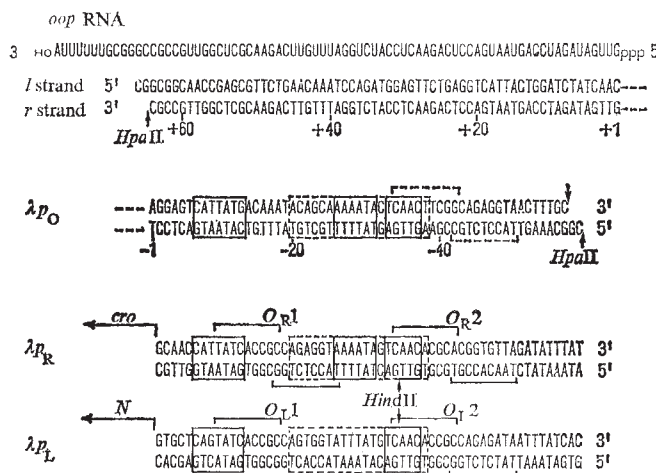


Fig. 4 Comparison of the sequence of the promoter *p_O* with the promoters *p_R* and *p_L* of phage λ . The sequences of the *p_R* and *p_L* promoters were taken from refs 18 and 32. The sequence predicted for the *oop* RNA is written on top of the transcribed section of the *Hpa*II F fragment; at the positions underlined the shown sequence deviates from the RNA sequence reported in ref. 7, as discussed in the text. The start points of transcription of the genes adjacent to *p_L* and *p_R* are indicated by horizontal arrows at the left ends. The boxes at left show the RNA polymerase binding sequences²¹ of the three promoter regions. The solid and dashed boxes further to the right point out sequences of total or partial (AT versus GC) homology between the three promoter regions. The presumed repressor binding sites in the *p_R* and *p_L* promoter regions^{18,32} are indicated by solid brackets, a similar sequence in the *p_O* promoter region by dashed brackets. The positions of the *Hind*II sites in *p_R* and *p_L* are also shown. The *p_R* promoter is given as the inverse of its usual orientation in λ DNA.

single C residue replaces an octanucleotide GCCGCCUU of the reported sequence (positions 46 to 53 of ref. 7). (3) Finally there are two C residues proximal to the AU dinucleotide at positions 33-34, and only one C residue distal to it, instead of the reverse.

This latter change is in agreement with the absence of an *Eco*RII cut from the *oop* DNA region (DNA pregrown under *mec* conditions¹⁹; unpublished observation from this laboratory). The CCUGG of the original sequence, which is the RNA analogue to one of the two recognition sequences of the *Eco*RII restriction enzyme²⁰, is now replaced by the sequence UCUGG in positions 34 to 38 (Fig. 4). As a consequence of the revisions given here, the entire sequence of the *oop* RNA is now predicted to consist of 77 rather than of 81 nucleotides.

In the promoter model put forward by D. Cribnow²¹, a promoter comprises three essential elements covering about 40 base pairs of DNA: a 'recognition' sequence, a 'binding' sequence, and an RNA initiation point. Five or six base pairs separate the binding sequence from the initiation point and a distance of about 20 base pairs is postulated between the binding and the recognition sequences. All the nine promoters the sequences of which have been published (see refs 21 and 22) show strong homology in a seven base pairs region which is believed to define the binding sequence^{21,23}. The *p_O* promoter region presented in this paper fully confirms this rule as indicated by the boxed sequence of Fig. 4, six base pairs ahead of the start point of transcription. While the binding sequence has now been well accepted, there is still uncertainty about the basic structure of the RNA polymerase recognition site. Comparison of the *p_R* and *p_L* promoters with a promoter for *E. coli* RNA polymerase in SV40 DNA (ref. 18) and of the G2 and G3 promoters of phage fd DNA with the same SV40 promoter²² revealed a five base-pair homology between these promoter sequences in the regions of the presumed recognition sites. The sequence of the *p_O* promoter region presented in Fig. 4 substantiates these findings. If one aligns these promoter sequences relative to their common binding sequences (and not

to their RNA initiation points), a homologous hexanucleotide sequence emerges:

λp_O	5	AGTTGA	3
λp_R		TGTTGA	
λp_L		TGTTGA	
SV40		TGTTGT	
fdG2		TGATGC	
fdG3		CGTTGT	

This sequence is placed always 20–25 nucleotide pairs ahead of the binding sequence (19–24 in the case of the fdG3 promoter). As has already been noticed^{21,22} the *lac* and the *tyrosine tRNA* promoter sequences do not fit into this group. Obviously, more sequence data are necessary in order to identify possible additional groups of basic recognition sequences. (Due to a different adjacent nucleotide (Fig. 4) the p_O promoter DNA is just missing to also contain a *HindII* site (GTPyPuAC) as do the promoters λp_R and λp_L in this area.)

The λ promoters p_R and p_L overlap with the serial operator sequences in O_R and O_L (refs 18 and 32). Whereas the structural features of O_{R1} (or O_{L1}) seem to be absent from the p_O promoter sequence, an operator-like sequence can be detected in the p_O promoter region, which is in exact register with the O_{R2} and O_{L2} operators (indicated by dashed brackets in Fig. 4). This sequence could be expected to act as a repressor binding site, but no reduction of the *oop* RNA transcription rate upon the presence of repressor has been observed *in vivo*⁸. In addition, no binding other than to the O_R and O_L fragments has been observed with λcI repressor and *HpaII* digestion mixtures of λ DNA (ref. 24) and $\lambda dvh93$ DNA *in vitro* (unpublished observation from this laboratory). Since binding sites for the *cro* gene product in O_L and O_R apparently overlap with *cI* repressor binding sites, an alternative explanation for the operator-like sequence in the p_O promoter region would be to function as an interaction site for the *cro* protein. This possibility remains to be tested directly, although several mutations of phage λ have been mapped in this part of the *ori* region which show an increased sensitivity against *cro* (ref. 25). To identify other possible significant sequence structures, work is in progress to extend the known part of the p_O promoter region.

We thank Drs W. Gilbert and A. Maxam for kindly providing us with a detailed protocol on their methods for strand separation and for degradation of labelled polynucleotides with dimethylsulphate and hydrazine before publication. We thank Miss S. Baars and Mrs E. Schieffmayr for technical assistance. Helpful criticism of the manuscript by Dr R. Hausmann is gratefully acknowledged. This work was supported by a Promotions-stipendium from the Studienstiftung des deutschen Volkes to G.S. and by grants from the Deutsche Forschungsgemeinschaft to G.H. and H.K.

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letters to nature

X-ray flare in 3U 0833–45 the Vela pulsar

DURING an extended observation with the Ariel V Sky Survey Instrument (SSI) a flare has been detected from the X-ray source 3U 0833–45, generally associated with the Vela pulsar. Details of the SSI, which covers the energy range 2.4–18 keV, are given elsewhere¹. The flare occurred during the first part of 24 July 1976, reaching a peak intensity at ~ 0300 UT of 11.9 ± 1.7 counts s^{-1} , a factor of three above its normal (Ariel V) level, and lasting ~ 0.5 d. Figure 1 shows the light curve of the event, and it is interesting to note a possible precursor to the main flare, occurring approximately 2 d earlier. No similar event or measurable variability has been seen in observations of 3U 0833–45 with the SSI over the previous 20 months. A typical run of data from August 1975 with 3U 0833–45 in its normal (quiescent) state is shown in Fig. 2. These lower flux values have been summed in 0.7 d bins for clarity in the figure.

3U 0833–45 is believed to be associated with the pulsar PSR 0833–45 (ref. 2) itself probably a remnant of the Vela SN explosion³. Pulsed emission at both hard⁴ and soft⁵ X-ray energies have been claimed for this pulsar, although, as yet, efforts to confirm these results have proved unsuccessful^{6–8}. It appears that the earlier results were incorrect or that the pulsed component is highly variable.

The chance of an unknown field source appearing briefly in the $0.7^\circ \times 10^\circ$ (FWHM) collimator of the SSI and coincident with the Vela pulsar in the scanning direction (offset $\sim 0.1^\circ \pm 0.2^\circ$) cannot entirely be ruled out. However, consideration of the frequency of such transient events found by Ariel V makes this very unlikely. Accepting, then, that the observed flare is indeed from the pulsar and taking a distance of $460 pc^{10}$ the total emitted energy (2–10 keV), assuming the spectral shape of Kellogg *et al.*², of the main and precursor flares is $2.9 \pm 0.3 \times 10^{38}$ erg and $1.2 \pm 0.3 \times 10^{38}$ erg respectively.

Mapping of the Vela region with Ariel V (A. Smith, unpublished) has confirmed the result first obtained from an analysis

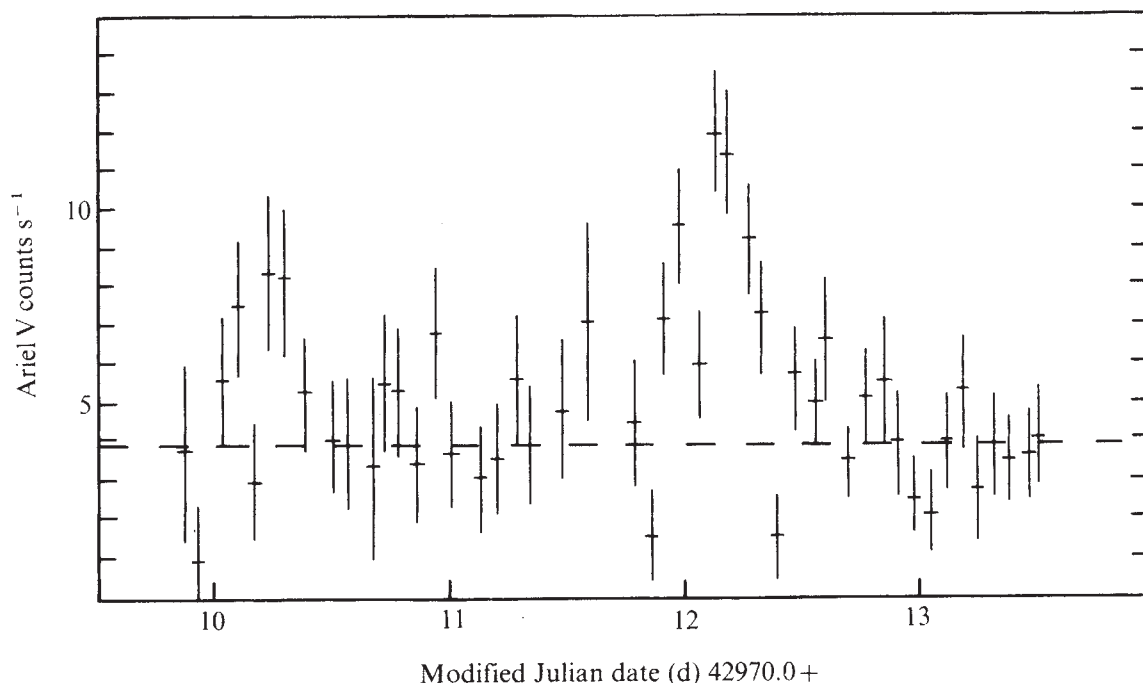


Fig. 1 Single orbit observations of 3U0833-45 during July 22-25 1976. Error bars are $\pm 1\sigma$. The broken horizontal line represents the normal flux for this source.

of Copernicus X-ray data by Silk¹¹, that the emission region around the Vela pulsar is extended. This extension is consistent with the normally steady X-ray emission of 3U 0833-45 and it is probable that the variable component now associated with the pulsar is generally undetectable and, correspondingly, that the variability of this point source component is much greater than a factor of three. This conclusion offers the possibility that the large pulsed component found by Harnden and Gorenstein⁴ was indeed correct and occurred during a period of enhanced emission. The likelihood of a short rocket observation coinciding with such a flare can be estimated from the previous SSI data, some 600 orbits of which spread over 20 months detected no similar flares. The estimated probability of a flare coinciding with one of six reported observations is $< 10\%$.

The 0.5 d timescale of the present X-ray flare may give a clue to its origin. Unfortunately no timing data was available during the observations and therefore it is not known whether the X rays were pulsed at the 89 ms period of PSR 0833-45. However, the absence of a concurrent change in the radio frequency ($\Delta\omega < 5 \times 10^{-10}\omega$, Manchester, personal communication) argues against the pulsar being the source of the flare. Equally, the short time scale rules out a major change in the X-ray

emission of the extended nebular source (diameter of 30 arc min ≈ 12 light years at 460 pc). The most probable source of the X-ray flare appears to be a redistribution of the magnetic field and particle flux in the magnetosphere of the pulsar. Scargle and Pacini¹² have discussed this possibility for the Crab Nebula to explain the occurrence of pulsar 'spin-ups' and 'spin-downs' and the considerably greater energy associated with the concurrent wisp activity.

The energy stored in the magnetosphere of PSR 0833-45 may be of the order of the magnetic energy of the neutron star: $\sim B_0^2 a^3$ (B_0 is the surface field and a the stellar radius). For $B_0 \sim 2 \times 10^{12}$ gauss (ref. 13) and $a \sim 10^6$ cm, the stored energy $\sim 4 \times 10^{42}$ erg. Beyond this region of magnetic trapping current models, for example Rees and Gunn¹⁴, envisage a relativistic wind flowing out radially to a shock boundary, where the outflow pressure is balanced by the gas pressure of the surrounding nebula. A release of energy from the pulsar magnetosphere, producing a temporary enhancement in the wind, would then give rise to enhanced synchrotron emission on striking the shock boundary. Although it is difficult to estimate the radius of this shock in Vela (no wisps seen, pressure in outer nebula uncertain), scaling from the Crab Nebula

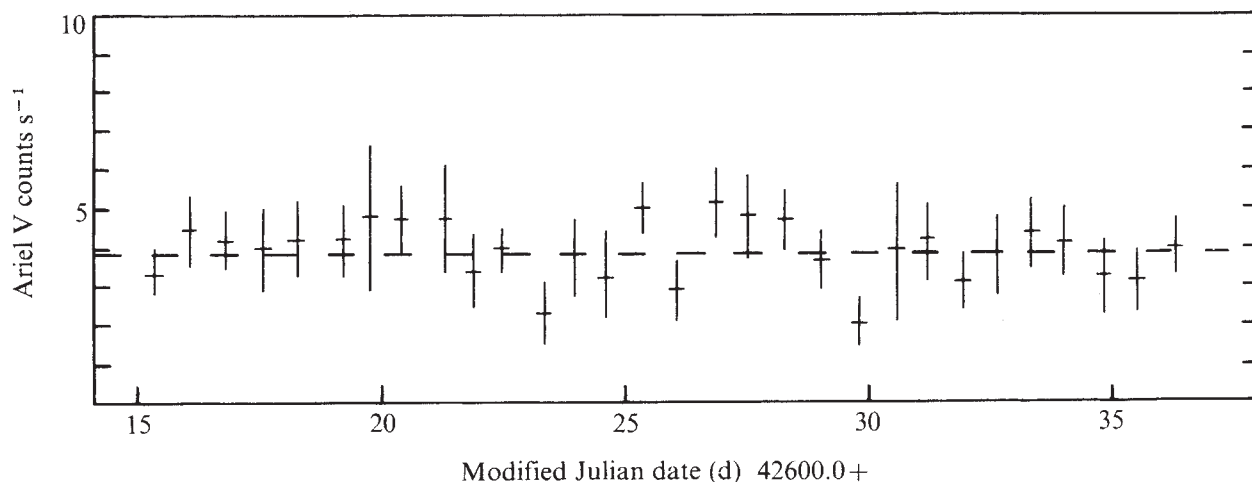


Fig. 2 0.7 d summed observations of 3U0833-45 during August 1975.

according to the ratio of mean pulsar losses (60:1), suggests $R_s \sim 5 \times 10^{16}$ cm for Vela. The observed flare timescale of ~ 0.5 d (10^{15} light s) may be satisfied if only part of the shock boundary is affected or, perhaps more likely, the beaming of the synchrotron X rays restricts the observed emission to that arising from the fraction of the shock surface beaming radiation in our direction.

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The missing intercloud medium and spiral galaxies

It is the purpose of this letter first to point out that several independent lines of evidence seem to indicate that the 'intercloud medium' does not exist in its usually quoted state with density $\gtrsim 0.1$ cm $^{-3}$ and temperature $T \sim 10^4$ K. Rather, a hot, tenuous medium ($n \lesssim 10^{-2}$ cm $^{-3}$, $T \sim 10^6$ K) seems more consistent with observations in the neighbourhood of the Sun. That such might be the situation has been previously suggested (see refs 1 and 2). A mechanism for producing such a medium has been proposed by Cox and Smith³. Second, this letter points out several implications of such a "missing intercloud medium" on the large-scale structure of spiral galaxies.

The lines of evidence pointing to the preponderance in the solar vicinity (within 1 kpc) of an intercloud medium having $n \lesssim 10^{-2}$ cm $^{-3}$ and $T \sim 10^6$ K (which we will call "Phase III" gas) are as follows: (1) Steigman, Strittmatter, and Williams¹, studying ultraviolet interstellar absorption lines, find no correlation between distance to the illuminating star and absorption line strengths for lines which arise from 'classical Phase II' ($n \gtrsim 0.1$, $T \sim 10^4$ K) intercloud gas. Instead, Phase II absorption line strengths are correlated with spectral type, indicating that circumstellar rather than interstellar Phase II gas is being observed. Column densities and ionisation states are shown to be consistent with circumstellar gas conditions one would predict from stellar wind theory.

(2) Ubiquitous interstellar O VI absorption is observed². The absorption strength is apparently correlated with distance to the illuminating star, while the radial velocities of the star and interstellar line are uncorrelated. The O VI ion is expected to be present in the ultra-hot Phase III medium, but not in the 10^4 K Phase II medium.

(3) Very stringent upper limits ($n_{\text{HI}} < 0.02$) exist on the neutral hydrogen density in the directions of two hot white dwarfs detected by the Apollo–Soyuz EUV experiment at wavelengths of 50–600 Å. (refs 5, 6). Detection of a third source

identified with another hot white dwarf, by another rocket-borne EUV experiment⁷, has recently placed a similar upper limit on n_{HI} in a third direction. In each of the three cases, the upper limits refer to averages over distances of about 75 pc. Even more dramatic, therefore, may be the non-detection by Copernicus of interstellar Lyman α absorption in the spectra of seven B stars located in the galactic plane at distances of up to several hundred parsec⁸, particularly if we follow ref. 4 and attribute many or all of the detections of interstellar Lyman α absorption in spectra of other stars observed by Copernicus to circumstellar material. It is important to note that these low neutral hydrogen densities cannot be explained as being due to highly ionised Phase II gas, since this explanation would violate the upper limit on electron densities derived from pulsar dispersion observations⁹ ($n_e < 3 \times 10^{-2}$). The low upper limits for both n_{HI} and n_e are of course consistent with the Phase III picture.

(4) When an expanding supernova remnant encounters a region of very low density, the remnant may disappear as a recognisable entity. If large fractions of the interstellar medium are in Phase III, this effect should cause a deficit of large radius supernova remnants. Jones¹⁰ has analysed the observations of remnant sizes and concludes up to 80% (by volume) of the interstellar medium is Phase III.

(5) An interstellar medium primarily composed of Phase III gas will have a galactic scale height ~ 1 kpc. Therefore, both the temperature and distribution of the proposed Phase III interstellar medium are consistent with the soft X-ray background^{11,12}.

(6) It is also interesting to note that the "observed" mean density of the gas which is purported to be the Phase II intercloud medium is inconsistent with the amount of matter traversed by, and lifetime of, galactic disk cosmic rays.¹³

(7) New 21-cm observations of the interstellar medium in the direction of bright high-galactic-latitude continuum sources (J. M. Dickey, G. E. Salpeter, and Y. Terzian, personal communication) seem to provide no evidence for the existence of a pervasive Phase II medium, although the presence of such a medium has been inferred from older observations of the same type.¹⁴ An 'intercloud' ($T_p > 500$ K) contribution to the emission and absorption profiles observed definitely exists, but appears to be correlated in velocity with the cloud ($T_p \sim 60$ K) contribution. Much of the 'intercloud' contribution also must come from warm gas of a few hundred degrees (J. M. Dickey, E. E. Salpeter and Y. Terzian, personal communication). These observations are completely inconsistent with the strict pressure-equilibrium two-phase model, and in fact seem to provide no evidence for the existence of any interstellar material not associated with clouds.

If one assumes that the mechanism which produces the ultra-hot interstellar medium in the solar neighbourhood of our Galaxy is that proposed by Cox and Smith³ (heating by shock waves from supernovae), then we can estimate the likelihood of also finding the Phase III medium in other spiral galaxies. Cox and Smith³ define a porosity, q , which is related to the fraction of the interstellar medium occupied by tunnels of very hot Phase III material

$$q \propto R n_0^{-1.7}$$

where R is the supernova rate and n_0 is the mean interstellar gas density. We take relative values of R (for various spiral types) from Tammann,¹⁵ and assume relative values of n_0 are given by the relative values (for the various spiral types) of the ratio of H I mass to total galactic mass.¹⁶ We find that values of q in the Sa, Sb, and Sc galaxies are all within 20% of the local value in our Milky Way, that is

$$0.8 \leq \frac{q(\text{Sa, Sb, Sc})}{q(\text{Milky Way})} \leq 1.2$$

It therefore seems that a Phase III interstellar medium (with embedded cooler clouds and circumstellar shells) could be a

general phenomenon in spiral galaxies. This has significant implications for theories of star formation in spiral arms that invoke the mechanism of a galaxy-wide, density-wave driven shock wave in the Phase II interstellar medium.

Large scale galactic shocks and corresponding filamentary spiral structure can form in the interstellar gas only if the gas is driven strongly enough to force the velocity component normal to a spiral arm, $w_{\perp}$, to oscillate about its unperturbed value $w_{\perp 0}$ and achieve transsonic values. Studies (see refs 17 and 18) which estimate the relative speed, $w_{\perp 0}$, of the density wave pattern and the interstellar medium suggest typical values $w_{\perp} \leq 25$ –50 km s⁻¹. Simultaneously the effective acoustic speed, a , is ~ 7 –12 km s⁻¹ if the gaseous component is largely Phase II gas, and the formation of strong shocks in this situation is rather natural.¹⁹

On the other hand, if the intercloud medium is predominantly 'Phase III' gas, as it seems to be in the solar neighbourhood, the effective acoustic speed, a , is ~ 100 km s⁻¹, and the formation of strong shocks is rather unlikely except in extreme circumstances of severe forcing. This implies that either the shock wave picture of star formation in spiral arms needs to be abandoned for galaxies with predominantly Phase III intercloud medium, or that density waves in such galaxies are strongly non-linear. (Strom *et al.*²⁰ have studied two galaxies which have spiral patterns in the old population stars, but lack the usual hot, young stellar population spiral arm tracers. We suggest these may be two examples of galaxies which have high enough porosity to preclude the formation of shocks and consequent star formation.)

Galaxies with Phase III gas throughout both arm and inter-arm regions seem likely to be quite uncommon, however. This is because the Phase III gas is formed primarily in the supernova-rich spiral arms and the cooling time for this gas is shorter than the time it takes the gas to travel between spiral arms (B. Smith, personal communication). It seems more probable, therefore, that Phase III gas dominates the intercloud medium in most spirals only near spiral arms, where most supernovae occur. The Phase III gas then cools to a Phase II medium in the interarm region. This newly cooled medium could then experience a shock on encountering the next density wave enhancement (indeed, the skewed character of spiral arm 21-cm profiles seems to imply shocks)²¹. The shock wave would produce more young stars which would result in supernovae which would in turn re-heat the interstellar medium to a Phase III temperature, thus completing the cycle. Such a situation would significantly affect the pressure and density of gas in the plane as a function of azimuthal angle, perhaps leading to an explanation of the observed lack of strong compressional effects in the spiral arm non-thermal continuum emission²². Of course for this type of picture to be consistent with all the observations in our Galaxy the Sun must lie near or within an active region of star formation and supernovae, where Phase III gas can be generated, such as a major spiral arm or a secondary feature or spur.

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Limit on the frequency of intergalactic supernovae

We shall turn the simple observation that "there are no intergalactic supernovae" into a quantitative statement for the Coma cluster of galaxies. In this cluster, where at least 30% of the total luminosity lies outside the regions normally associated with individual galaxies^{1–3}, the absence of intergalactic supernovae can be used to derive restrictions on the nature of the luminous intergalactic material, be it a diffuse collection of stars, a background of low surface brightness dwarf galaxies, or some other form of luminous matter.

Relevant data on the supernovae within 3° of the Coma cluster centre are collected in Table 1. Most of the data came from the list of Sargent *et al.*⁴, except the data on SN 1976 (*IAU Circular* no. 2921, 1976; the radial velocity from ref. 5), and the type of the parent galaxy of SN 1962i (ref. 6).

Two supernovae listed in Table 1 fall well within the Holmberg radius of the foreground galaxy NGC 4725 (SNe 1940b, and 1969h). As a luminosity class I Sb-galaxy⁷ this object is expected to be a good supernova producer. In addition, it is very unlikely that the coincidence in position is a product of chance and that these supernovae belong to the Coma cluster background: within 3° from the cluster centre there are only five foreground galaxies brighter than $m_{pg} = 15.0$ mag (ref. 8); their combined Holmberg area covers $\approx 3 \times 10^{-3}$ of the total area. Even when allowance is made for fainter, unidentified foreground galaxies, the probability of a Coma cluster supernova falling by chance within the Holmberg radius of a foreground galaxy is smaller than 10^{-2} . One supernova of Table 1 (SN 1968h) belongs to an anonymous galaxy, the high redshift of which lies well outside the typical velocity interval of Coma cluster galaxies⁹ and identifies this galaxy as a background-object. The supernova is most probably physically associated with this galaxy: its central distance is only 0.03 Holmberg radii. We will exclude these foreground and background objects from our discussion.

No radial velocities are available for the parent galaxies of SNe 1962i and 1963m. We adopt these galaxies as Coma cluster members because the probability of cluster membership is high for any galaxy in the field considered, and because the morphological appearance of the galaxies agrees well with the hypothesis of cluster membership. The parent galaxy of SN 1962i is quite faint: although it seems somewhat brighter than $m_{pg} = 19$ mag assigned by Zwicky *et al.*⁶, it could be a distant background galaxy. However, the fact that the SN 1962i was brighter at discovery (16^m.5) than its parent identifies the latter as a dwarf galaxy¹⁰, and hence as a very probable Coma cluster member. Our sample contains ten Coma cluster supernovae; of these, five have been assigned types: four as type I and one as peculiar⁴.

Table 1 Supernovae within 3° of the Coma cluster centre

Supernova designation (1)	Suspected parent galaxy (2)	Right ascension* (3)		Declination* (4)		Type of parent galaxy (5)	$V^\dagger$ (km s ⁻¹) (6)	$m_{pg}^\ddagger$ (7)	Supernova type (8)	$D_{EW}^\S$ (arc s) (9)	$D_{NS}^\S$ (arc s) (10)	$R_H^\parallel$ (11)
		h	min	°	'							
1940b	N4725	12	48.0	25	46	S(B)b	1114	10.2	II	95E	118N	0.22
1950a	I4051	12	58.5	28	17	E1	4932	14.8	I	2W	13N	0.23
1959b	N4921	12	59.0	28	08	Sa	5459	13.7	pec	16E	48S	0.36
1961d	Anon	12	48.3	28	06	E0	7638	14.8	I	32E	12S	0.93
1962a	Anon	13	04.3	28	08	S0	6137	16.0	I	11W	7N	0.25
1962i	Anon	13	00.5	27	47	E	—	19.0	—	8W	7N	0.56
1963c	Anon	12	55.3	28	09	E	6050	15.3	I	5W	6N	0.32
1963m	Anon	12	55.5	28	20	S0p	—	16.3	—	2W	7S	0.21
1968b	N4874	12	57.2	28	14	S0	7171	13.7	—	12W	1N	0.08
1968h	Anon	12	55.9	27	24	Sb	12050	16.6	I	9W	2N	0.03
1969h	N4725	12	48.0	25	46	S(B)b	1114	10.2	I	18E	10N	0.20
1973f	N4944	13	01.5	28	28	S0	7009	13.3	—	27E	3N	0.26
1976	N5004a	13	08.7	29	50	SBa-b	7163	15.3	—	7W	18N	0.28

*Epoch 1950.0.

†Heliocentric radial velocity.

‡Apparent magnitude of parent.

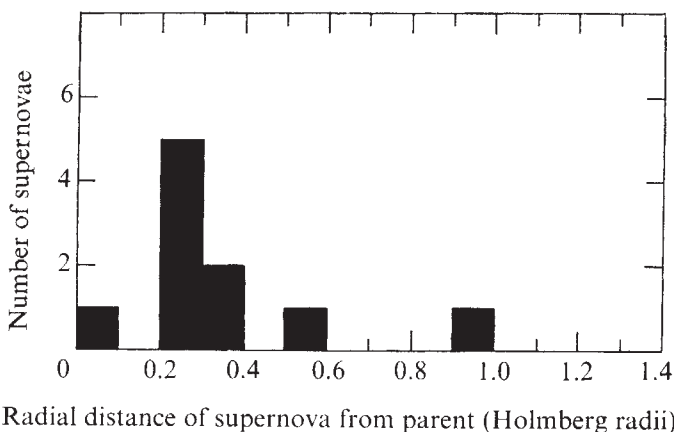
§Distances of supernova from centre of parent (east-west and north-south).

||Radial distance of supernova from parent in units of the Holmberg radius, derived as described in text.

The radial distances given in Table 1, column 11, were calculated by assuming that the outer isophote of each galaxy is an ellipse with the major and minor axis given by the Uppsala diameters¹¹. Galaxies not appearing in the Uppsala list were measured independently, and these measures were converted to the Uppsala scale. Using the Uppsala diameters and position angles and the supernova distances of Table 1, we determined the ratio of the supernova distance to the distance of the Uppsala isophote at the same position angle. These radial distances were converted to distances in units of Holmberg radii¹² using a scaling factor determined from galaxies common to both lists. Figure 1 shows a histogram of these radial distances.

To estimate the supernova rates per unit luminosity in the Coma cluster, the following quantities are needed: the luminosity in galaxies within our 3° radius, the effective number of search years, and the relative luminosity in spiral and elliptical galaxies. For the luminosity within a 3° radius, we take the value $L = 9.6 \times 10^{12} L_\odot$ derived from the data of Thuan and Kormendy² using the model proposed by King¹³ to extrapolate to 3°. ($H_0 = 55 \text{ km s}^{-1} \text{ Mpc}^{-1}$ is assumed throughout.) This value agrees within a factor of two with the values found by other observers^{14,15}. Barbon¹⁶ estimates the efficiency of the Palomar supernova search to be 90% between 1957 and 1967. Our list contains six supernovae discovered during this time, and hence one could conservatively estimate that our sample of ten Coma supernovae corresponds to 15 effective search years.

Fig. 1 Histogram of radial distribution of supernova in the Coma cluster galaxies.



This number is consistent with the fact that the Coma field was surveyed at Palomar alone during 96 different months in the years 1958–1971 (ref. 4). However, the uncertainties in this estimate are difficult to evaluate, and we consider this only as a reasonable indication of the effective search time. The ratio of luminosity in ellipticals to that in spirals can be estimated from the data for the brighter cluster members provided by Rood *et al.*¹⁴, and we will take $L(\text{E/SO}):L(\text{Spirals}) = 6:1$.

The above data imply the following supernovae rates for the eight supernovae in E/SO galaxies and for the two supernovae in spirals

$$\begin{aligned} v_e(\text{E/SO}) &= 6 \pm 2 \text{ SNU} \\ v_e(\text{Spirals}) &= 8 \pm 5 \text{ SNU} \end{aligned}$$

where the units of SNU (supernova units) are $\text{SNe } (100 \text{ yr})^{-1} (10^{12} L_\odot)^{-1}$. These numbers are dependent on the assignment of types to the parent galaxies. If we had adopted the types from Maza and van den Bergh⁷, the frequency in E/SO galaxies would have been decreased by about a factor of two and that in spirals increased by roughly the same factor. For comparison, the rates in nearby galaxies given by Tammann¹⁷ are

$$\begin{aligned} v_{NB}(\text{E/SO}) &= 25 \pm 10 \text{ SNU} \\ v_{NB}(\text{Sa}) &\approx 20 \text{ SNU} \\ v_{NB}(\text{Sc-Irr}) &= 140 \pm 20 \text{ SNU} \end{aligned}$$

The rates for the Coma cluster galaxies may be less than for the nearby sample. This may not be statistically significant, but if it were, it might have been anticipated in view of the fact that the searches tend to discriminate against supernovae in the centres of more distant galaxies⁴. If we use the results of Sargent *et al.* on supernova frequencies as a function of the apparent magnitude of the parent galaxy as the basis for an extrapolation from Tammann's nearby galaxy sample to the distance of the Coma cluster, we would expect to find a still lower supernova rate (by a factor ~ 2) in the Coma cluster than in fact we do. Thus we conclude that the region of the Coma cluster has been quite carefully surveyed and that the galaxies are producing supernovae at about the same rate as the nearby galaxies.

An upper limit to the intergalactic supernova rate can be derived from the value for the intergalactic luminosity. We again use the data of Thuan and Kormendy² and the model of King¹³ to derive the value $L = 5.0 \times 10^{12} L_\odot$ as the luminosity outside the Holmberg radii of the galaxies. This estimate agrees with that of de Vaucouleurs¹. Since none of the ten

supernovae in our sample have occurred outside the Holmberg radius of the parent, we derive a limit on the intergalactic supernova rate by assuming one intergalactic supernova

$$v_{IG} < 1 \text{ SNU}$$

This rate is more than six times less than our observed rate in Coma cluster E/SO galaxies and more than ten times less than the rate in nearby E/SO galaxies.

An independent limit on the relative efficiency of the intergalactic material for producing supernovae can be derived by the following argument. We observe no supernovae outside the Holmberg radius in ten examples. This would occur with 50% probability if only 6.7% of the supernova producing matter were outside the Holmberg radius. In fact, 34% of the total luminosity is exterior to the Holmberg radius and, hence, this intergalactic material is at least five times less efficient in producing supernovae than the material in galaxies.

Our entire discussion has assumed that the intergalactic region has been as carefully surveyed as the regions around the individual galaxies themselves. For the Coma cluster, we believe that this may be true since the cluster is rather compact, and the empty regions between galaxies are relatively small so that an observer would not be likely to miss supernovae even at large distances from the galaxies. As already mentioned, the searches tend to discriminate increasingly against central supernovae in more distant galaxies, and this effect should tend to increase the relative frequency of outlying supernovae. On the other hand, there is no quantitative way of estimating the search efficiencies in the intergalactic regions, so that our conclusions on the intergalactic supernovae rates could be taken, at worst, as limits on the relative survey efficiency. Clearly, we do not support this view, but cannot be quantitative and must rely on what we know and hear about how the searches are conducted. At least in the case of the Palomar search, the plates have been uniformly blinked over the entire region (C. T. Kowal, personal communication).

With the above caveat, we conclude that the intergalactic medium is deficient in supernova progenitors by a factor of ≥ 5 . This holds independent of the nature of the luminous material, be it the effect of overlapping haloes of E/SO galaxies, the light of undetected dwarf galaxies, or of any other origin.

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Demonstration of upper and lower Newtonian fluid behaviour in a pseudoplastic fluid

THE most common type of non-Newtonian fluid is the pseudoplastic fluid. The pseudoplastic fluid is characterised by a constant viscosity at very low shear rates, a viscosity which decreases with shear rate at intermediate shear rates, and an apparently constant viscosity at very high shear rates. That is

$$\lim_{\dot{\gamma} \rightarrow 0} [\tau/\dot{\gamma}] = \eta_0 \text{ (zero shear viscosity)} \quad (1)$$

and
$$\lim_{\dot{\gamma} \rightarrow \infty} [\tau/\dot{\gamma}] = \eta_\infty \text{ (infinite shear viscosity)} \quad (2)$$

The apparent viscosity defined by

$$\eta = \tau/\dot{\gamma} \quad (3)$$

then decreases with shear rate from η_0 to η_∞ and the pseudoplastic fluid exhibits a lower and upper region of Newtonian behaviour. Examples which illustrate the zero shear viscosity and the decreasing viscosity region for pseudoplastic fluids are very common and include almost all polymer melts and solutions. η_∞ is generally not well defined. Often the data available only approach the high shear rate limit and η_∞ is either determined by extrapolation or is set equal to zero which is a suggested theoretical value. Data which illustrate the three regions of pseudoplastic behaviour are rare and indeed are not quoted in the standard reference texts on rheology. The purpose of this communication is to present such data for a polymer solution and to compare the results to the models which are available for pseudoplastic fluids.

Expressions of varying complexity have been proposed to model pseudoplastic behaviour. The Meter model¹ contains η_0 , η_∞ and two other parameters, τ_m and α .

$$\eta = \eta_\infty + \frac{\eta_0 - \eta_\infty}{1 + (\tau/\tau_m)^{\alpha-1}} \quad (4)$$

τ_m is the shear stress at which the viscosity η has dropped to $\frac{1}{2}(\eta_0 + \eta_\infty)$ and hence is another measurable quantity. α is a measure of the rate at which η is reduced from η_0 to η_∞ and can be determined from the slope of a plot of $(\eta_0 - \eta)/(\eta - \eta_\infty)$ against τ on log-log coordinates, providing both η_0 and η_∞ are known. If $\eta_\infty \ll \eta_0$ then $\tau_m = \tau_{1/2}$, the shear stress at which the viscosity has dropped to $\frac{1}{2}\eta_0$, and the Meter model reduces to the Ellis model².

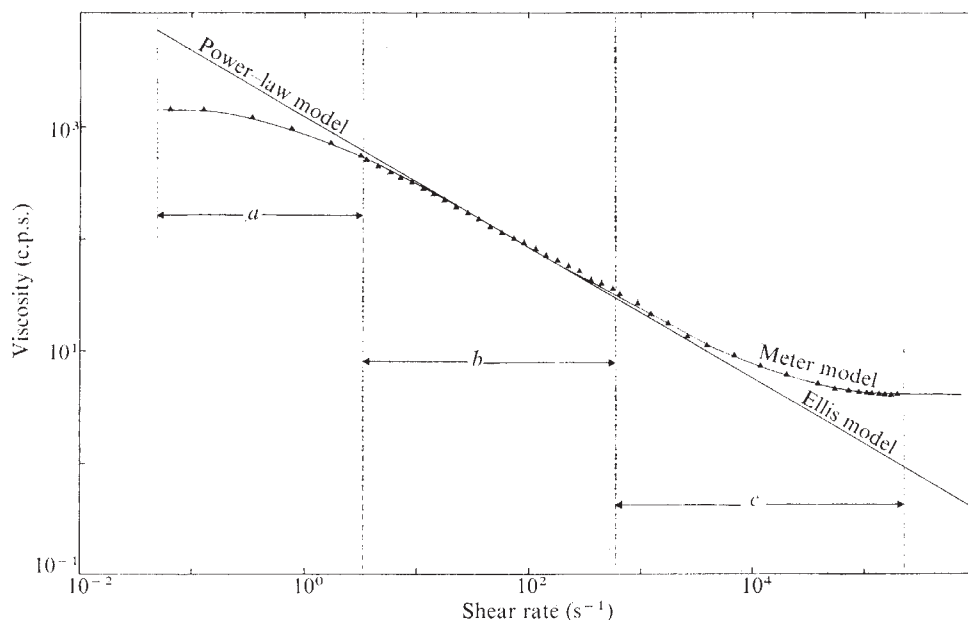
$$\eta = \frac{\eta_0}{1 + (\tau/\tau_{1/2})^{\alpha-1}} \quad (5)$$

which in turn shows power-law behaviour at high shear stresses

$$\eta = K\tau^{1-\alpha} = \eta_0(\tau_{1/2})^{\alpha-1}\tau^{1-\alpha} \quad (6)$$

Three instruments, a Brookfield viscometer, a Weissenberg rheogoniometer, and a gas-driven capillary rheometer were used to make shear stress-shear rate measurements over a large enough shear rate range to illustrate upper and lower Newtonian behaviour. A 0.4% weight polyacrylamide solution was used. The Brookfield viscometer with a cylindrical spindle of length 6.36×10^{-2} m and diameter of 1.87×10^{-2} m was used to measure the shear stress as a function of shear rate at low shear rates. Relative to the diameter of the spindle, the fluid medium in which the spindle was immersed was infinite in size. End

Fig. 1 Illustration of upper and lower Newtonian behaviour for a 0.4% polyacrylamide solution. *a*, Brookfield viscometer; *b*, cone and plate viscometer; *c*, capillary viscometer.



corrections were less than 10%. For this condition the shear stress and the shear rate on the surface of the spindle are given by ref. 5.

$$\tau_b = \frac{T}{2\pi R_b^2 L} \quad (7)$$

$$\gamma_b = \frac{4\pi N}{n''} \quad (8)$$

where T = measured torque; R_b = radius of spindle; L = length of spindle; N = rate of revolution of the spindle (r.p.s.); n'' = slope of a log-log plot of τ against N for each value of τ .

A 2°1'26'' cone was used with a 0.075 m diameter plate in the Weissenberg rheogoniometer. For the small cone angle employed the shear stress and hence the shear rate do not vary in the gap between the cone and plate and the shear stress and shear rate are related to the torque exerted on the plate and the rate of rotation of the cone by⁶

$$\tau = \frac{3T}{2\pi R^3} \quad (9)$$

and
$$\dot{\gamma} = \Omega/\alpha \quad (10)$$

where R = radius of the platens; Ω = angular velocity of the cone; α = cone angle.

A gas-driven capillary rheometer was used for the high shear rate region. The tube internal diameter was 5.29×10^{-4} m and its length was 0.3789 m. Exit and entry pressure losses are negligible for such a high L/D capillary tube and the wall shear stress and wall shear rate are defined in terms of the measured pressure drop (Δp) and volumetric flow rate by^{5,6}

$$\tau_w = \frac{R\Delta p}{2L} \quad (11)$$

$$\gamma_w = \frac{3n+1}{4n} \left(\frac{4Q}{\pi R^3} \right) \quad (12)$$

where n is the slope of the log-log plot of τ_w against $4Q/\pi R^3$ at each value of $4Q/\pi R^3$.

Equations (7), (8), (9) and (10) were then used to reduce the torque as a function of angular velocity data, while equations (11) and (12) were used to express the pressure drop flow rate data obtained with the capillary rheometer in terms of the wall shear stress and wall shear rate. The results obtained with the three instruments are shown in Fig. 1 where the apparent viscosity defined by equation (3) is plotted against the shear rate. The polyacrylamide solution showed no appreciable shear degradation at the high shear rates employed in the capillary rheometer.

The results in Fig. 1 cover almost six decades of shear rate and the reproducibility between the various instruments is excellent. Upper and lower Newtonian behaviour is clearly illustrated with $\eta_0 = 1425$ cP and $\eta_\infty = 4.0$ cP. Also shown on Fig. 1 are the Meter, Ellis, and power-law model fits of the experimental data with $\tau_m = 1.215$ N m⁻², $\alpha = 2.43$ and $\tau_i = 1.211$ N m⁻². Notice that the Meter model is an excellent representation of the data over the entire shear rate range, the Ellis model is inadequate for shear rates in excess of 250 s⁻¹ and that the power-law model is strictly valid only for 25 s⁻¹ $\leq \dot{\gamma} \leq 250$ s⁻¹. The results also clearly illustrate the importance of measuring the flow properties of a pseudoplastic fluid in the range of shear rates for which the data is to be employed and further, illustrate the importance of choosing the appropriate instrument and flow geometry for making these measurements. Clearly the measurement of the flow properties of a pseudoplastic fluid or for that matter any non-Newtonian fluid in a shear rate range other than that where the data is employed is a futile exercise.

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Exact modelling of cubic lattice permittivity and conductivity

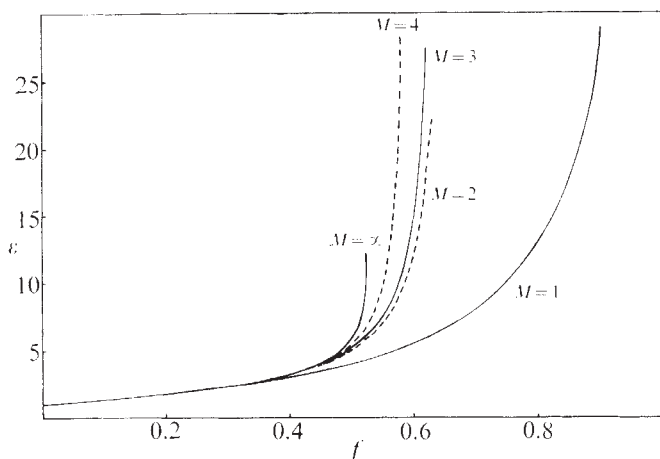
A PROBLEM of practical importance in a wide variety of situations is that of calculating the permittivity (or equivalently, the conductivity) of simple cubic arrays of spheres embedded in another material. Applications occur in, for example, the analysis of optical properties of inhomogeneous films¹, dielectric dispersion in mixtures², the behaviour of artificial dielectrics³, the study of lunar soil samples⁴ and in vision research⁵. Although the problem is an old one, and has attracted the attention of some of the great names of physics (for example, J. C. Maxwell⁶ and Lord Rayleigh⁷) it seems that a completely satisfactory theory has not existed until the present time. This is shown by the fact that in the most critical case, that of perfectly conducting spheres, none of the existing theories give the expected divergence at the critical volume fraction ($f_c = \pi/6$) at which the spheres touch. We have extended Lord Rayleigh's treatment and present a theory which gives the correct behaviour for all volume fractions.

The first order (dipole) solution to the problem is known as the Lorentz-Lorenz equation or the Maxwell Garnett equation

$$\epsilon = 1 - \frac{3f}{[(2 + \epsilon_1)/(1 - \epsilon_1)] + f} \quad (1)$$

where ϵ , ϵ_1 are, respectively, the permittivities of the resultant medium and of the spherical particles (both measured relative to the permittivity of the matrix); f is the volume fraction of the particles. The range of f over which equation (1) is accurate is not clear. This is a question of some importance in that controversy exists as to the cause of discrepancies between equation (1) and optical measurements made on samples with $f > 0.3$. Some authors attribute the discrepancies to variation of ϵ_1 with particle radius⁸ while others invoke various distributions of particle shape and orientation⁹. We consider that, before these two hypotheses can be meaningfully tested, proximity (higher order multipole) effects should be taken into account.

Fig. 1 Permittivity ϵ of a simple cubic array of perfectly conducting spheres as a function of volume fraction f . The order of the theory is indicated. $M = 1, 2$, and 3 correspond respectively to the theories of Maxwell Garnett, Rayleigh (corrected) and Meredith and Tobias. $M = 4$ corresponds to equation (3) and $M = \infty$ to the exact curve.



In his classic paper⁷ Rayleigh gave a second order treatment, with the aim of determining the region of validity of (1) for the cubic lattice. He took into account the effect of octupoles as well as dipoles (only 2^{2M-1} poles with M integral being allowed by symmetry). It is unfortunate that Rayleigh made two simple errors in the derivation of his final formula. The first of these (the omission of a factor of π) was corrected by Runge¹⁰; the second (the omission of a factor of $2/5$ which arises in a partial differentiation of his equation (61)) is corrected here.

Lord Rayleigh's treatment was extended by Meredith and Tobias¹¹ to include the effects of poles of order 2^5 . We have derived a formula which includes the effects of poles of order 2^7 :

$$\epsilon = 1 - 3f/D \quad (2)$$

$$\text{where } D = T_1^{-1} + f - b_1 T_5 f^{14/3} - c_1 T_7 f^6$$

$$- a f^{10/3} \frac{[1 - c_2 T_5 f^{11/3} + c_3 T_5^2 f^{22/3}]}{[T_3^{-1} + b_2 f^{7/3} - c_4 T_5 f^6]} \quad (3)$$

Here

$$T_n^{-1} = \frac{\epsilon_1 + [(n+1)/n]}{1 - \epsilon_1} \quad (4)$$

$$\text{and } a = 1.3045 \quad (5)$$

$$\left. \begin{aligned} b_1 &= 0.01479 \\ b_2 &= 0.4054 \end{aligned} \right\} \quad (6)$$

$$\left. \begin{aligned} c_1 &= 0.1259 \\ c_2 &= 0.5289 \\ c_3 &= 0.06993 \\ c_4 &= 6.1673 \end{aligned} \right\} \quad (7)$$

Full details of the derivation of these constants (which included the re-evaluation of certain sums) will be presented later. Equation (3) yields the formula of Meredith and Tobias if c_1 to c_4 are replaced by zero. The corrected equation of Lord Rayleigh results if in addition b_1 and b_2 are replaced by zero.

Table 1 f_c and $f_{\max}$ as a function of M , the number of terms in the multipole expansion

M	f_c	$f_{\max}$
1	1.000	0.30
2	0.66505	0.41
3	0.64469	0.43
4	0.59250	0.46
10	0.54260	0.48
25	0.5279	0.515
50	0.5248	0.522
100	0.5239	

We have generalised the formalism in such a way as to permit its programming to any desired order of the multipole field expansion. Figure 1 shows the comparison of results provided by our programme for theories of increasing order M . Note that only the highest order theory has its divergence at the correct value of f . This point is illustrated more explicitly in Table 1, which also shows the upper limit on f , $f_{\max}$, at which the theory of order M is correct to within an absolute tolerance of 5×10^{-2} .

While a great deal of experimental information exists on disordered arrangements of spheres, we know of only two sets of measurements on simple cubic arrays of perfectly conducting

particles^{3,11}. These are compared with the results of theory in Fig. 2. The theoretical curve has been tested for convergence and is accurate to better than 1%. The complete agreement between theory and experiment is evident. It is interesting to note that the experimental measurement made closest to the touching point, with $f = 0.5161$, differs from theory by less than 0.1. We have shown numerically that to obtain this agreement we must include multipoles of order up to 2^{29} . This is a tribute to the remarkable accuracy of the measurement.

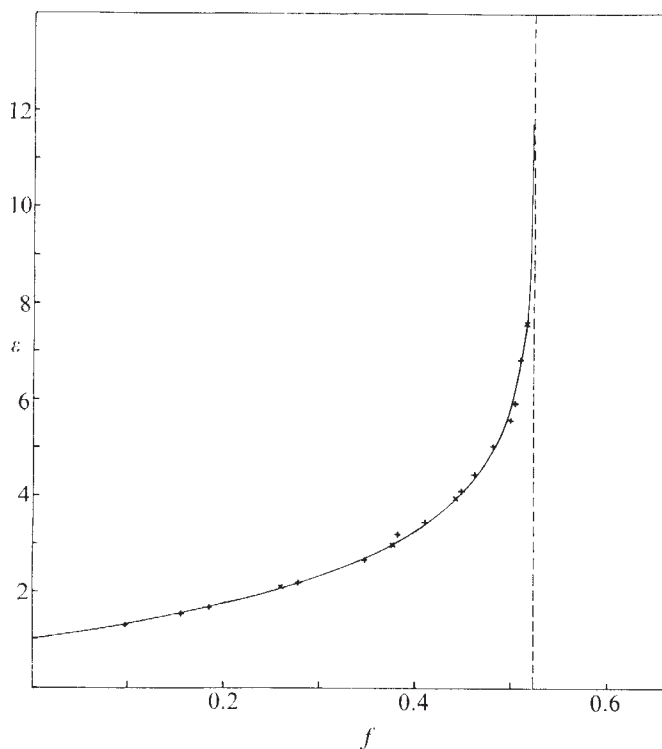


Fig. 2 Experimental measurements of Kharadly and Jackson (+) and of Meredith and Tobias (x) compared with the exact theoretical curve ($M = \infty$) of Fig. 1. The vertical dashed line corresponds to $f = 0.5236$.

Although we have discussed here only results pertaining to real permittivities of the spheres, the theory is general and its application to complex permittivities will be discussed later. We also envisage its extension to the close-packed lattices with the aim of devising a formula for close-packed random arrays. It is evident from the results above that such a formula is necessary to supplant the Maxwell Garnett equation (1) for dealing with random arrays having a volume fraction f greater than about 0.3.

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Surface circulation in Liverpool Bay

CONCERN over the influence of industrial and domestic effluents on the coastal waters of Liverpool Bay has led over the last decade to a number of physical, chemical and biological surveys^{1–7}. Despite this intensive effort there is little that can at present be stated with confidence about the form and variability of the circulation (A. J. Bowen, T. R. S. Wilson and M. J. Howarth in ref. 1, vol. 3, pages 45–55). Conclusions derived from direct observations of residual currents (refs 2 and 3 and J. W. Ramster in ref. 1, vol. 2, pages 56–64) and indirect inferences from plankton⁴ and chemical distributions^{5–7} have combined to produce strong evidence in favour of either a clockwise^{2,4} or anticlockwise (refs 3, 5–7 and J. W. Ramster in ref. 1, vol. 2, pages 56–64) surface residual circulation.

Direct observations of current direction and speed can be obtained over protracted periods from either continuously recording current meters (A. J. Bowen, T. R. S. Wilson and M. J. Howarth in ref. 1, vol. 3, pages 45–55; and J. W. Ramster in ref. 1, vol. 2, pages 56–64) attached to fixed buoys or from radio-tracked parachute drogues⁸. The obvious difficulty here is the sparse spatial distribution of synoptic data that can be obtained on anything other than a local scale. An alternative approach to elucidate the direction if not the magnitude of the circulation is to make use of the distribution of salinity, temperature and conservative chemical characteristics of the waters. The potential of utilising the intrinsic and quite often unique chemistries of the source waters of Liverpool Bay in any quantitative sense has largely been ignored⁷. This presumably is a consequence of the relatively large number of source waters that may significantly contribute to the area, and hence the number of different tracers required for the solution of any mixing model, together with the large number of samples that would have to be collected in this area of highly heterogeneous water chemistry⁹ to provide comprehensive and unambiguous distributions.

Our objective has been to use the distributions of physical and chemical characteristics to determine the surface residual circulation pattern in Liverpool Bay. Dissolved concentrations of silicate, phosphate, nitrate, nitrite and ammonia were chosen as potential tracers in conjunction with salinity and temperature. The chemical species clearly do not meet the conservative prerequisite of a natural tracer during periods of sustained primary production and hence can only be used during the winter period. They can, however, be analysed by continuous on-line techniques^{9,10} which provide greater definition of distributions and allow unambiguous interpretations that are not often possible with more traditional methods based on sampling at discrete stations.

Continuous records of the surface variability of these seven characteristics were obtained during a number of cruises on a comprehensive grid through Liverpool Bay during the winters of 1974 and 1975. Detailed descriptions of the observed distributions will be reported elsewhere. The number of contributory source waters and end member

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properties on each cruise were obtained by a combination of principal component analysis^{9,11} and examination of the envelopes enclosing 21 X - Y diagrams generated by sequential plots of coincident data from the continuous records. On all cruises so far conducted the number of major sources identified has been smaller than the number of characteristics monitored, permitting a number of mixing model solutions from the same data set simply by varying the combination of tracers used.

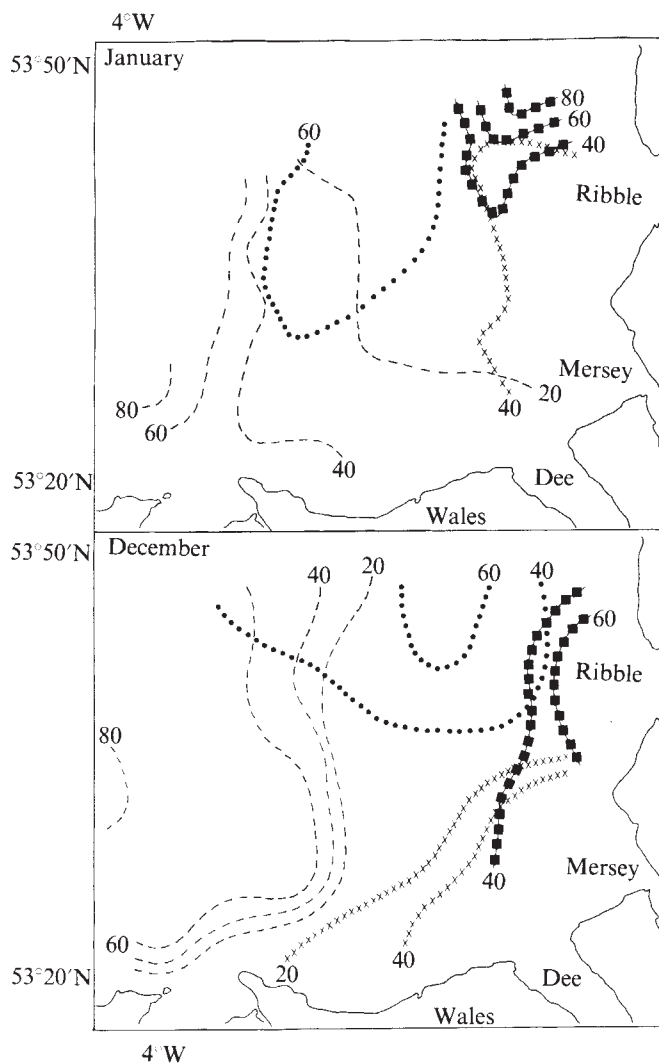


Fig. 1 During both January and December four major water types were identified. Mixing model solutions, showing percentile distributions are illustrated, both derived from salinity, temperature and silicate data. --- Western boundary water; ●, northern boundary water; × and ■, fresh water.

Model solutions for the cruises so far conducted have produced two distinct extremes, observed during January and December 1975. The solutions are illustrated in Fig. 1, which shows that (1) in January, western boundary water encroached well into Liverpool Bay along the North Wales coast, in contrast to December when a marked physical discontinuity was observed at approximately 3°50'W. (2) In December a significant contribution from fresh water sources persisted along the North Wales coast, whereas in January a northerly transport of these waters was apparent, (3) in both January and December, a significant contribution to the waters in the centre of the bay originated from

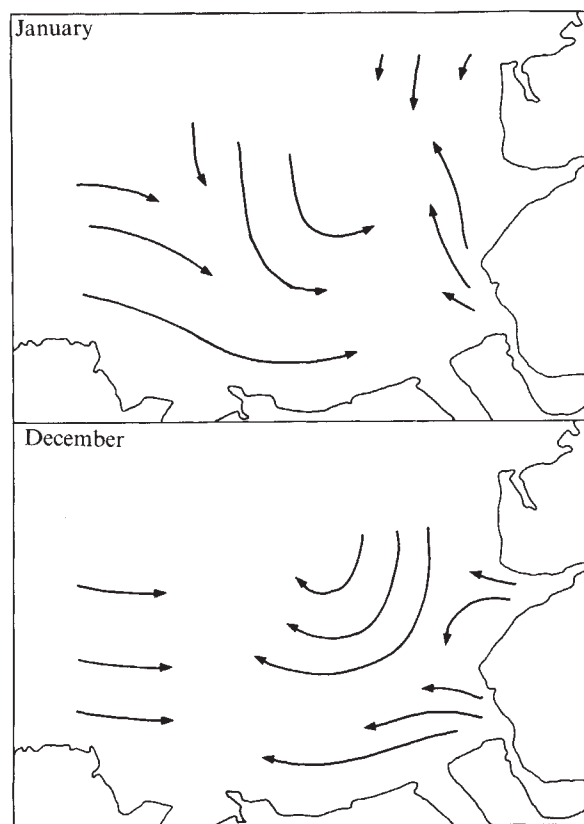


Fig. 2 Suggested surface circulation patterns in Liverpool Bay, January and December, 1975.

the northern boundary. Figure 2 shows the suggested circulation patterns. This novel approach to elucidation of the circulation pattern of Liverpool Bay demonstrates that either an anticlockwise or a clockwise circulation of the surface waters may be experienced during the winter period.

Of particular significance is the sharp contrast in the spatial distributions of potential pollutants associated with each circulation mode. Surface distribution maps exhibited high pollutant concentrations over an extensive sea area during periods of sustained clockwise circulation. We conclude that the replacement time of the fresh waters (particularly from the River Mersey with its associated industrial and domestic effluents) discharged into Liverpool Bay during periods of clockwise surface circulation is significantly greater than when the motion is anticlockwise. The possibility of deleterious consequences in the marine regime resulting from the terrestrial discharges is intimately related to the factors which initiate and subsequently maintain the clockwise circulation mode. Elucidation of these factors is the objective of continuing cruises.

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Cyclonic ring formation at the polar front in the Drake Passage

THE first summary of geographical locations of the antarctic convergence (polar front) by Mackintosh¹ showed that >50% of the instantaneous locations of the front differed from the mean location by as much as ± 50 km. Early hydrographic section work could not resolve the temporal or spatial fluctuations in the horizontal structure of the

antarctic and subantarctic water masses⁵, is most clearly revealed in vertical sections of temperature as that from our data shown in Fig. 1. South of the frontal zone there is a temperature minimum layer between 100 and 300 m. As noted by Gordon⁶, this feature is insulated from seasonal heating at the surface. Within the frontal zone the minimum deepens abruptly and erodes splitting into multiple extrema of interleaving antarctic/subantarctic waters. Although numerous definitions of the polar front have been suggested⁷, the operational definition used in our analyses is the presence of a well defined temperature minimum layer as delineated by the 2 °C isotherm. The selection of the 2 °C isotherm is somewhat arbitrary, but it does provide a convenient, consistent, and objective method for defining the position of the southern edge of the frontal zone.

Synoptic maps of the front have been produced with the combined data from the two vessels. While some artistry is involved in smoothing the observations, the horizontal density of measurements is sufficient to resolve the structure. A composite of these maps, Fig. 2, indicates that between March 10 and 24 a large meander developed in

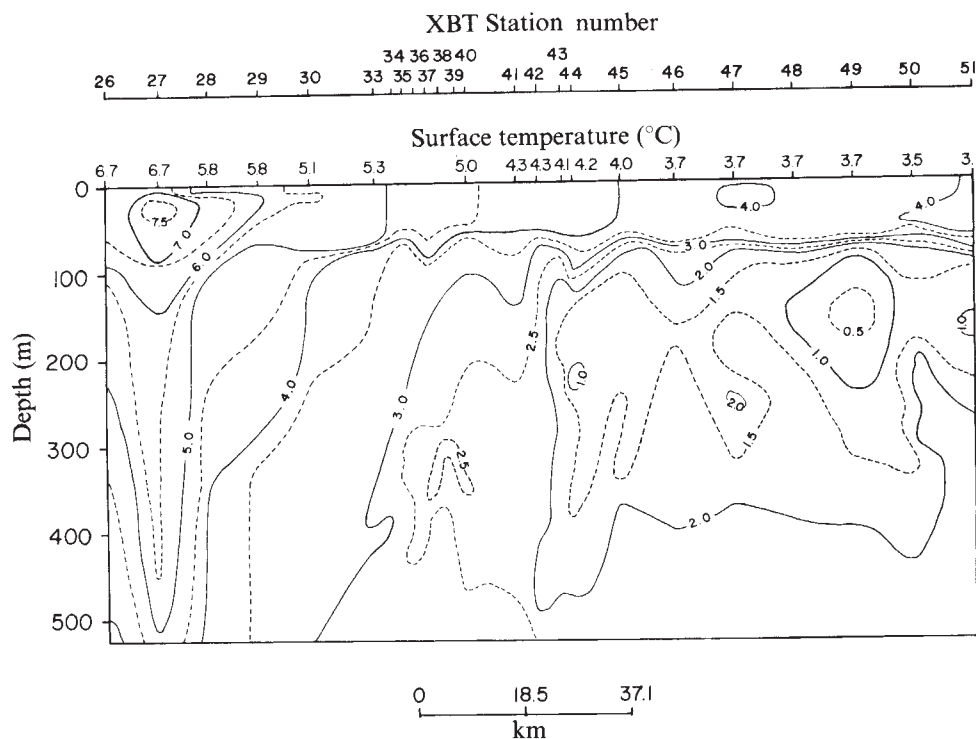


Fig. 1 XBT section across the polar front. Contours are in °C. Starting and ending positions for section are: XBT 26 (56° 44.8' S, 65° 30.4' W), XBT 51 (59° 1.95' S, 63° 2.5' W).

front. During FDRAKE 75 Gordon² observed comparable fluctuations in the frontal position in the Scotia Sea but again the spacing between sections was too wide. During FDRAKE 76 (refs 3, 4 and T. M. Joyce *et al.*, unpublished) the small scale structure of the polar front was studied in the Drake Passage. Two ships, the RV Thompson (University of Washington) and the Chilean naval vessel AGS Yelcho, combined efforts in the program which took place during the onset of the austral autumn. We describe here the evolution of the polar front between February 29 and April 5, 1976, a time span of ~5 weeks.

The polar front, in reality a transition zone between

the polar front. This is verified by thermistor chain measurements (position shown in Fig. 2) from a subsurface mooring⁸ in the vicinity and by the distribution of surface properties (not shown). The meander subsequently pinched off between March 28 and 30 into a cold, cyclonic ring of ~60–80 km diameter which then drifted to the NE at a speed of ~10 cm s⁻¹. The ring formation process is reminiscent of that observed in western boundary currents such as the Gulf Stream⁹. Continuous profiles of temperature and salinity show that the antarctic water types inside the ring penetrate to a depth of 2,500 m (Fig. 3), suggesting that much of the water column is involved.

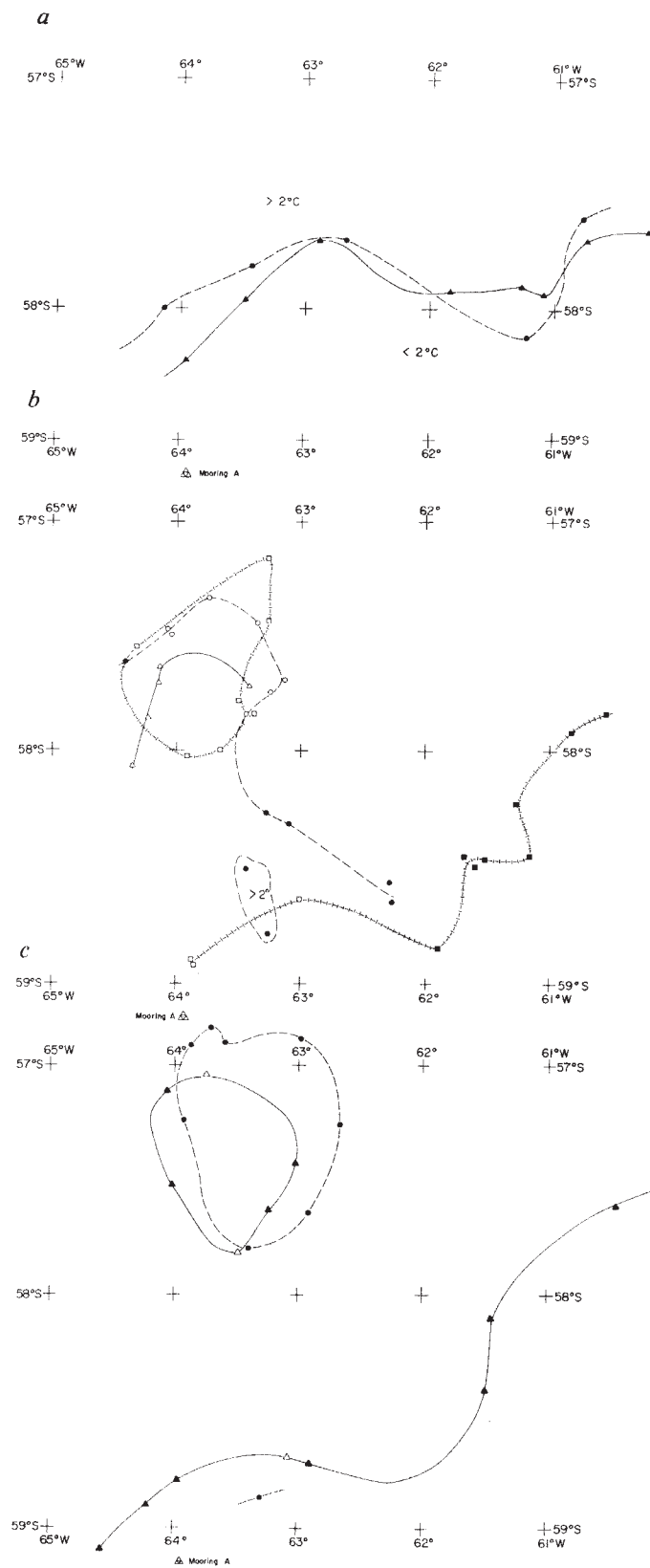


Fig. 2 Evolution of the polar front. Shown contoured is the boundary between antarctic waters and the interleaving regime of the frontal zone. Times are coordinated universal (CUT). *a*, February 29, 0130–March 5, 1830 Δ , —; March 8, 1330–March 9, 1540 $\circ$, — — —. *b*, March 16, 0000–March 20, 2359 Δ , —; March 21, 0000–March 26, 2359 $\square$, — — —; March 27, 0000–March 30, 2359 $\circ$, + + + +. *c*, March 31, 0000–April 3, 2359 Δ , —; April 4, 0230–April 5, 1315 $\circ$, — — —. Solid (open) symbols represent observations from the *Yelcho* (Thompson).

Subsurface neutrally buoyant floats equipped with fins to react to vertical water motion^{10,3}, were tracked acoustically from the *RV Thompson* during the period of the ring formation. The track of these floats (Fig. 4) indicates that three of the four vertical current meters (VCM) were entrapped in the ring while the fourth continued unaffected to the NE. Horizontal velocities of the floats were 30–40 cm s⁻¹. The depth of each VCM at launch and recovery is indicated in Fig. 4.

A series of CTD (conductivity, temperature, depth) stations to 3,000 was made directly through the ring by the *RV Thompson*. These data, together with nutrients and dissolved oxygen need to be analysed before a complete

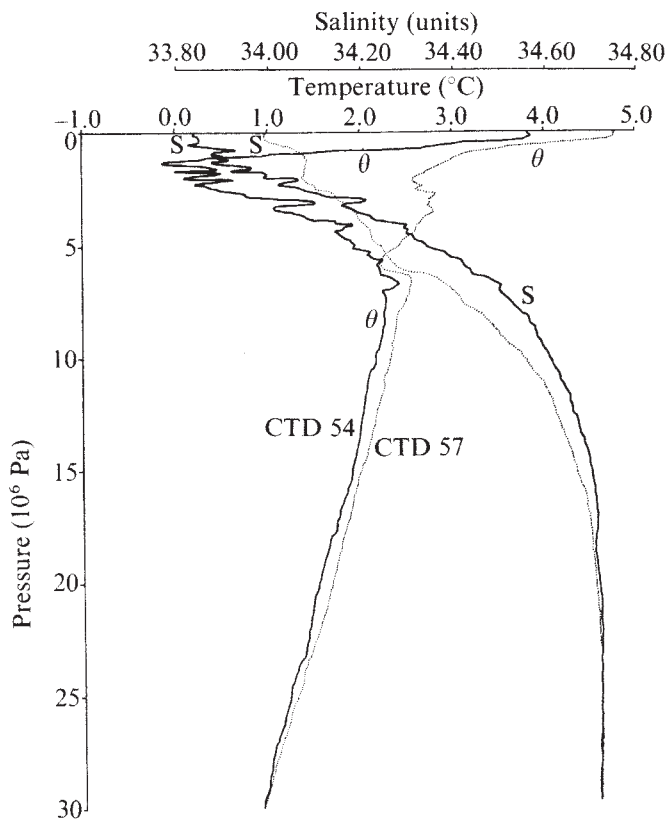


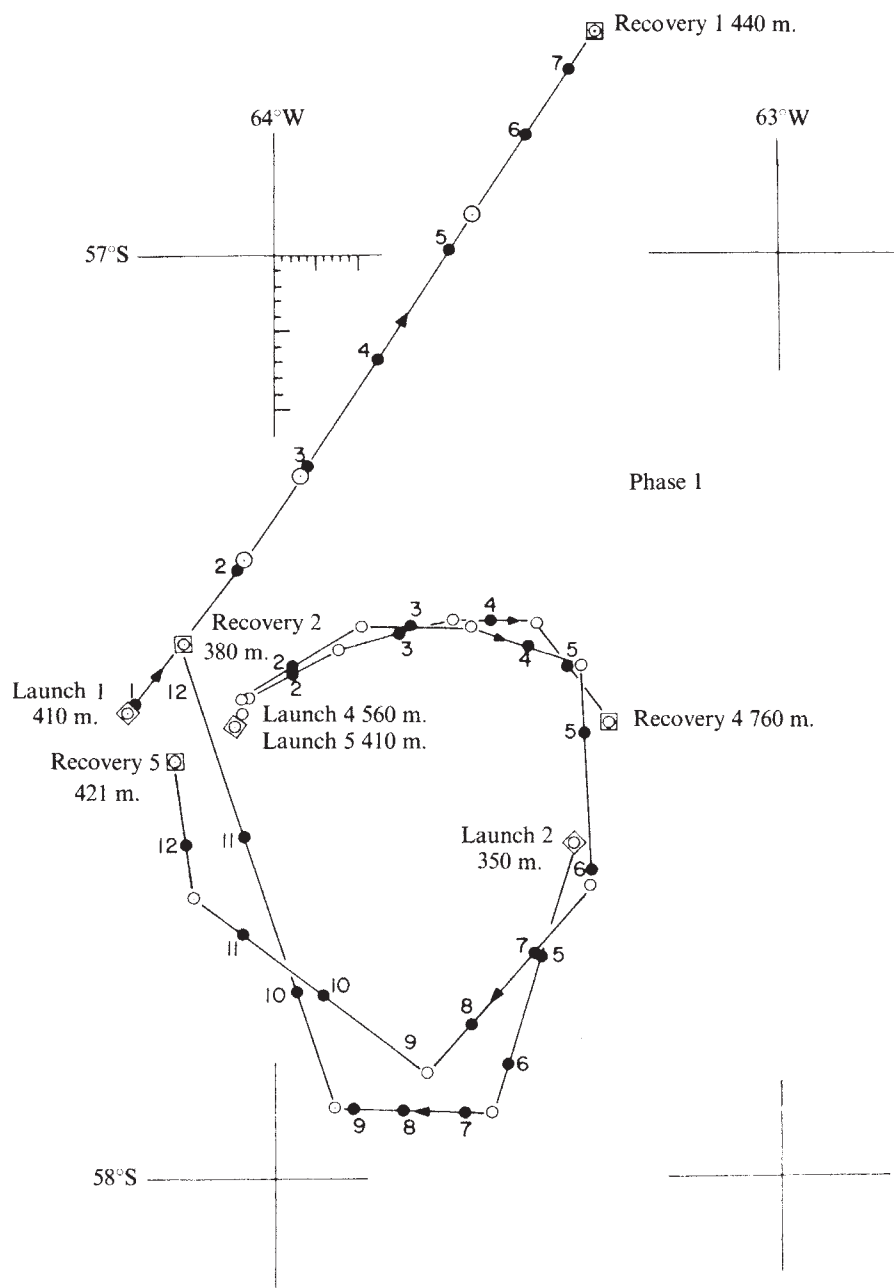
Fig. 3 Conductivity, Temperature, Depth (CTD) stations inside (54) and south, outside of the ring (57) but still north of the re-established polar front. Station locations are: 54: 57°39.9' S, 63°51.0' W, and 57: 57°59.9' S, 62°59.8' W.

description of the eddy properties (for example, circulation) can be determined. This work is in progress.

The process described here can potentially transport large amounts of antarctic or subantarctic water across the front and may be fundamental to the understanding of heat and salt transfers towards the polar region. If the cold ring is reabsorbed into circumpolar current, the net transfer of properties will be less than if the ring were to remain detached, slowly decaying with time. Downstream of the Drake Passage, the circumpolar current system is highly constrained by the Scotia Ridge¹¹ which has only two large openings with a sill depth >2.5 km. It is interesting to speculate upon the fate of our cold water ring when it drifts into this rather formidable topography.

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Fig. 4 Vertical current meter trajectories during cruise. Phase I was during the cold ring formation. The meander pinched off before March 30, when the float tracks began to close. □, launch; ○, recovery; ●, 12 h time marks starting 0000 March 24.



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¹²C/¹³C in cellulose and lignin as palaeothermometers

SEVERAL workers^{1–3} have attempted to retrieve palaeoclimatic information from the ¹²C/¹³C ratios in tree rings. Their use of whole wood, however, has been unsatisfactory, in that the extractives are isotopically much lighter than cellulose⁴ and vary considerably in content between heartwood and sapwood. Further, lignin is usually ~ 3‰ lighter than cellulose and it is well known from the extensive literature on the chemistry of wood that the lignin-to-cellulose ratio varies not only across one ring, that is, in late wood as compared to early wood, but up and down and radially across the tree⁵. The science of dendroclimatology is based on the observation that narrow rings represent a cold (or dry) year. In most conifers the amount of late wood remains relatively constant and it is the thickness of early wood that varies. A narrow ring would be expected to have a smaller proportion of early wood than a wide ring. The different proportions of lignin in the two rings would then lead

to a different isotopic ratio for the whole wood. If whole wood is used the $\delta^{13}\text{C}$ measurements will reflect the relative proportions of lignin and cellulose. We therefore separated the lignin and cellulose from the wood before making $\delta^{13}\text{C}$ determinations, hoping that the temperature coefficients of their production reactions might be large enough to enable the temperatures at which the carbon was fixed from the atmosphere to be measured. We also wished to investigate the possibility that the difference in $\delta^{13}\text{C}$ between the cellulose and lignin might reflect temperature.

Preliminary experiments indicated that any temperature effects would be small; probably towards the lower limit of current mass spectrometric techniques. We therefore studied the annual rings of a specimen of *Pinus radiata* (Monterey Pine) which had grown in Hamilton, New Zealand. This tree has the advantage of laying wood all year⁶, that is, over a temperature range of 10 °C. This enabled wood to be sampled that grew over a much wider temperature range than the range of post-glacial temperature fluctuations (perhaps 1–2 °C).

Fifteen samples over three annual rings were separated into cellulose and lignin using the conventional techniques of wood chemistry⁷. The samples were burned to produce CO_2 and this was measured in a Micromass 602C. The reproducibility of our measurements based on 12 separate combustions of the sample was $\pm 0.04\text{‰}$ (1 σ). From Fig. 1 it can be seen that there is a depletion in ^{13}C for late (winter) wood as compared with early (summer) wood for both cellulose and lignin. Keeling⁸ has measured the isotopic ratio of atmospheric carbon dioxide over the Pacific Ocean and shows that there is an annual fluctuation of $\sim 0.3\text{‰}$, the atmosphere becoming isotopically

heavier in the late summer and autumn. This effect was attributed by Keeling to the activity of land plants⁸. The annual change we measured is of different phase and an order of magnitude greater than the change in the atmosphere. Thus we consider that the variations of $\delta^{13}\text{C}$ shown in Fig. 1 are caused by changes in air temperature affecting one or more of the biochemical reactions leading to cellulose and lignin. During the year in Hamilton both the monthly mean maximum daily temperature and the monthly mean daily temperature vary through a cycle with an amplitude of 10 °C. Thus we obtain a temperature coefficient for both cellulose and lignin of at least $0.2\text{‰ per }^\circ\text{C}$.

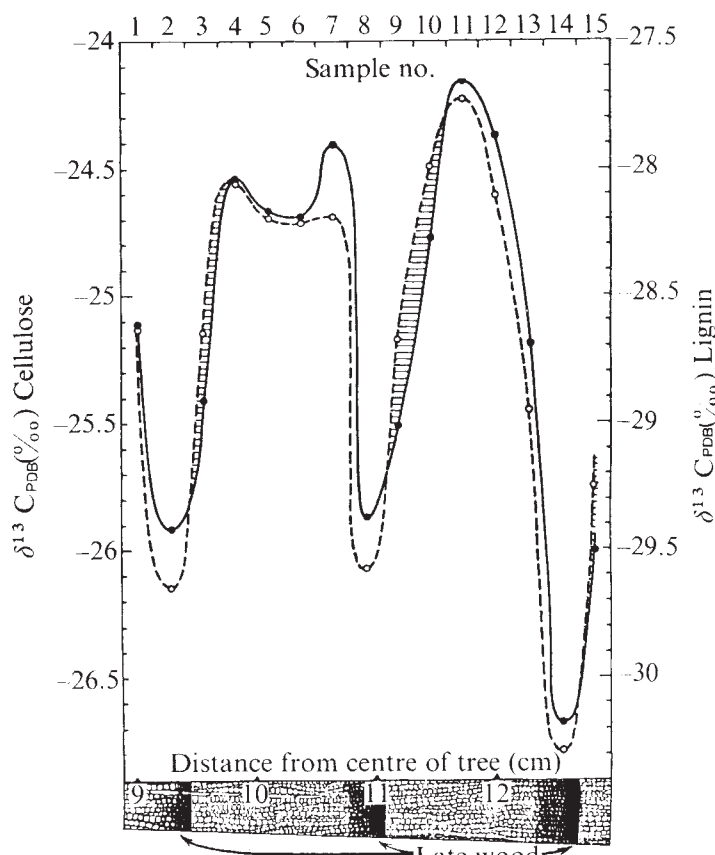


Fig. 1 Variation in the isotopic ratio of the carbon in cellulose (a) and lignin (b) across tree rings from *Pinus radiata*.

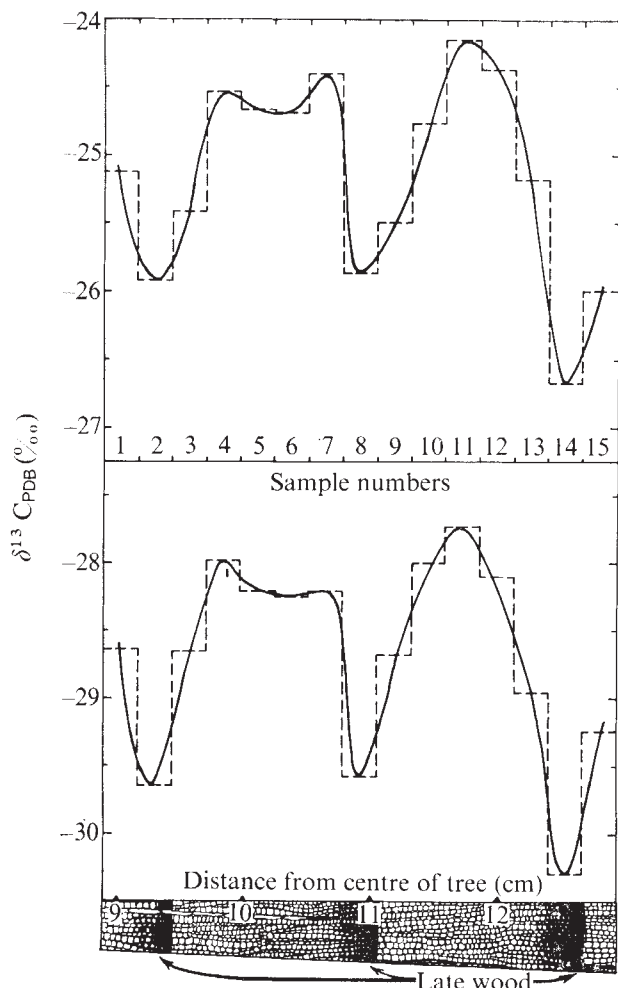


Fig. 2 Variations in the isotopic ratio of carbon across tree rings from *Pinus radiata*—a comparison between cellulose (—) and lignin (---).

Figure 2 presents the cellulose and lignin data superimposed. Several conclusions are evident. First, the amplitude of the $\delta^{13}\text{C}$ values of cellulose and lignin are very similar. This probably can be taken as evidence that the temperature dependent steps in the biochemical pathway leading to both cellulose and lignin are early in the sequence, probably in the leaves. Thus the temperature being monitored is that of the leaves rather than the trunk of the tree.

Second, although the cellulose and lignin curves are significantly different, these differences are probably too small to be of any use in palaeoclimatology with present mass spectrometric techniques. The third interesting result is the phase relationship between the cellulose and lignin curves. It is generally believed by plant physiologists that xylem cells are laid down and some time later lignified⁹. In Fig. 2 we can see the result of this secondary lignification, in that the lignin is significantly displaced in time and hence temperature with respect to the cellulose. This is excellent confirmation of the general interpretation given above.

If, as our results seem to indicate, the $\delta^{13}\text{C}$ values of cellulose and lignin vary with temperature, we may now ask if they will be of any use in determining past climate. Modern mass spectrometric techniques rarely measure $\delta^{13}\text{C}$ to better than 0.1‰ and few people have claimed to have made measurements with a

standard deviation of better than 0.06‰ (0.3 °C). Modern mass spectrometers (such as Micromass 602C) can measure a sample of pure CO₂ with an s.d. of 0.02‰. The problem is associated with combustion of the sample, and in particular, impurities that contribute to the mass 45 peak in the mass spectrometer and hence limit the accuracy. We have found that with very careful combustion, great care in removing any water (which can form COOH⁺ of mass 45) and by repeated sampling, one can make measurements with a precision of $\pm 0.02\text{‰}$ (1 σ), that is, measure temperatures to 0.1 °C. This would be adequate for palaeoclimatic studies provided one could assume that the $\delta^{13}\text{C}$ of the atmosphere has remained constant or is known.

This problem has been studied by Keeling^{8,10} and others. Clearly there is an effect arising from man's combustion of fossil fuels since the Industrial Revolution. There are also localised effects in the vicinity of large industrial centres, and seasonal effects which have been attributed to the activity of land plants^{8,10}. This latter effect would be averaged out if one sampled wood from a number of years. Another problem that must be avoided is that the atmosphere near the ground in a forest can be depleted in ^{13}C . This has been attributed to root and mycorrhizal respiration utilising photosynthates which are depleted with respect to the atmosphere. It has been observed that young plants growing in such a situation and therefore the internal rings of large trees are depleted in $\delta^{13}\text{C}$ (refs 1, 11, 12). Once the tree reaches maturity—perhaps because its foliage reaches the free atmosphere—the $\delta^{13}\text{C}$ content stabilises. Data from the inside rings of large trees will have to be treated with suspicion or the 'juvenile' effect—which is probably a smooth curve—corrected for. But, provided trees are chosen from suitable sites and corrections are applied for any man-made effect, it seems that $\delta^{13}\text{C}$ measurements on cellulose and/or lignin hold promise for obtaining past temperatures from sub-fossil wood. Since in some areas tree-ring records extend back annually for up to eight millennia, the prospect is exciting.

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Precambrian evolution in a stratified global sea

HARGRAVES¹ postulates that continents on Earth emerged from a circumglobal ocean perhaps as recently as 1.0–1.4 $\times 10^9$ yr ago. Without arguing the merits of the geological evidence supporting this theory, we suggest that the heuristic value and biological implications of this hypothesis are remarkable.

If such a sea existed some 3 $\times 10^9$ yr ago, then life must have originated in this environment. In conformance, then, with current theories concerning the conditions necessary for life's genesis² we would expect high concentrations of both reduced organic carbon compounds and nutrients such as phosphate. Although physical conditions in such an ocean during the formative stages are arguable, surface heating by solar radiation and wind mixing would, in all probability, produce a warm low density surface

layer. Without continents to obstruct horizontal oceanic currents or differentially heat the Earth's atmosphere, heat distribution on the planet's surface would be more uniform than at present. The high latitude generation of deep water would be less pronounced. While equatorial upwelling might still be expected, its magnitude is not clear and certainly the prominent upwellings now associated with continental margins would not exist. This primordial ocean, like a permanently stratified low latitude lake, would function essentially as two distinct subsystems—a warmer, wind-mixed epipelagic region and a cooler, poorly mixed, bathypelagic region. Once life began, nonconservative nutrients and reduced carbon would, as in present oceans, be removed from the surface waters by gravitational settling of organic remains.

Three implications emerge from these considerations. First, in the surface waters, evolutionary selection would favour cells with the high surface area-to-volume ratio essential for scavenging dilute nutrients. The food web, we suggest, was in gross outline comparable with that in the open oceans today³. Dissolved organic carbon compounds were anaerobically utilised by nano-plankton-sized saprobes and these in turn were preyed on by nano or small carnivores. With size restricted by nutrient concentrations, evolutionary selection for larger multicellular forms was absent and life remained at the unicellular level.

Second, we suggest that in light of the inverse relationship between body size and metabolic rate⁴ and the lower energy yield obtained in anaerobic fermentation⁵, primordial heterotrophs rapidly depleted the supply of reduced organic substrate in surface waters. On the assumptions that (1) initially the primordial ocean was 10% by weight reduced organic carbon⁶; (2) the epipelagic region extended to a depth of 100 m; (3) no reduced carbon was returned from bottom waters, and (4) the rate of organic carbon utilisation was comparable with that of heterotrophs in the oceans today, then reduced carbon reserves in surface waters could have been depleted within 250,000 yr. This suggests that in surface waters there was early evolutionary selection first for organisms able to utilise less reduced organic carbon⁷, and then for photo-autotrophs able to fix carbon dioxide. This argues for an early evolution of photosynthetic capability and the release of oxygen to the Earth's atmosphere and could account for the occurrence of chlorophyll in appreciable amount as early as 3 $\times 10^9$ yr ago⁸.

A third implication is persistence of anaerobic conditions in the bathypelagic region. Although a rate of photosynthesis comparable with that in the least productive regions of the present oceans could, in a period of 50,000 yr, yield a quantity of oxygen comparable with that in the present atmosphere, any buildup of atmospheric oxygen reserves would have little effect on the bottom waters. The continued input of reduced carbon from above, limited mixing, and the large volume of this lower region would militate against rises in oxygen concentration.

With the development of continental platforms able to obstruct horizontal ocean circulation, conditions in the primordial ocean would alter considerably. Upwellings developed along continental margins would elevate nutrient concentrations in at least limited regions of the photic zone. A consequent increase in both total energy fixed by autotrophs, and in cell size of autotrophs, would occur and in response to this latter change, the difference between carbon fixed by autotrophs and carbon utilised by autotrophs would increase. The net result would be an increase in the amount of energy available to heterotrophs and selection for metazoan forms. Platforms within 200 m of sea level may be required to initiate such upwelling⁹.

At the outset selection might be for free swimming ciliated or flagellated forms structurally similar to the larval stages of most para or metazoan phyla. But with locomotor ability increasing in proportion to surface area and mass increasing in proportion to volume, a shift from planktonic to benthic modes of life would ultimately occur. Soft bodied, sessile, filter feeders, and burrowing, detrital feeders characteristic of marine benthic and neritic sediment would quickly colonise the elevated continental platforms.

Finally, evolutionary selection for large multicellular forms would come when persistent land forms emerged and regions

comparable with the highly productive estuaries of today's Earth appeared. In response to the increases in nutrient concentration resulting from continental runoff, there would be selection for plants and animals able to withstand the rigours of a turbulent, near-shore environment. Large attached plants and hard-bodied animals evolved and, in a geologically brief period, colonised the newly emergent land forms.

Although the foregoing is only a sketchy outline, it is clear that if Hargraves is correct, we must rethink many ideas on biological evolution. Whereas conventional wisdom envisions life as slowly evolving until the early Cambrian and then blossoming to fill an array of previously sterile habitats, we would instead see evolution as a process rapidly generating forms to colonise each environment as it became available. The development of land masses and the evolution of life are viewed as intimately linked and the fossil record should provide dates for critical stages in Hargraves's chronology.

This vision of biological evolution in a stratified world ocean has important consequences in many other areas—among them the evolution of the terrestrial atmosphere and the origin of banded iron formation. For example, the Berkner–Marshall¹⁰ view that Precambrian faunal development was retarded by lack of atmospheric oxygen is no longer viable. An oxygenated atmosphere nearly 3×10^9 yr before present is more consistent with fossil¹¹ and carbon isotope data^{12,13}.

Precambrian banded iron formation may represent periods of pronounced upwelling which for some reason (too brief in duration?) did not yield significant evolutionary development. Ferrous iron from upwelling waters would be precipitated on mixing with oxygenated surface waters. Seasonal variations in the intensity of coastal upwellings are not uncommon and could account for the banded character of banded iron formation. This mechanism is not grossly unlike that envisioned by others (for example ref. 14).

In conclusion it should be noted that the concept of a circum-global sea, and the traditional view of early and persistent continents are mutually exclusive. Re-examination of data in the light of these different theories seems most appropriate.

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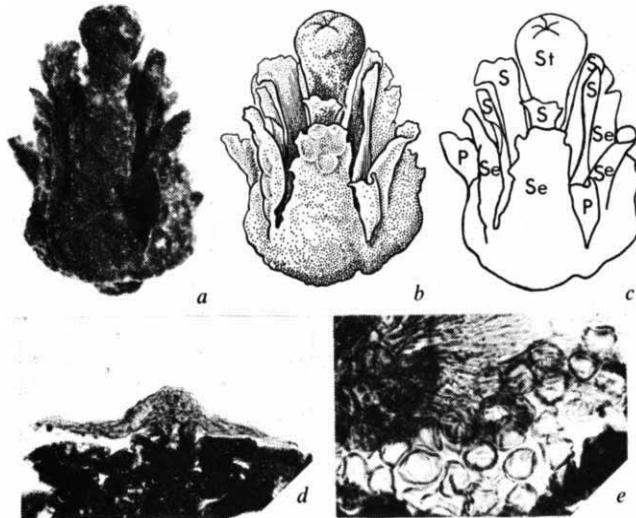
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be based on the comparative morphology of living forms. The discovery of the three-dimensionally preserved flower herein described, and its associated remains, is thus of considerable interest.

Upper Cretaceous plants have previously been described from ironstone concretions and sandy clays on the island of Martha's Vineyard, Massachusetts¹. Mr Clifford Kaye of the United States Geological Survey called my attention to a more recently exposed occurrence of highly fossiliferous, dark-grey, sulphide-rich lignitic clay on the cliffs at Gay Head, at the extreme western end of the island. This clay has been dated by pollen analysis as earliest Campanian in age (Upper Cretaceous, about 75 Myr old) and is the source of the recently described gymnosperm cones *Pityostrobus kayei* and *Pseudoaraucaria arnoldii*². Approximately 2,000 kg of this clay were collected, and subsequently disaggregated and sieved to concentrate the organic fraction. This was placed in hydrofluoric acid to remove adherent mineral matter, and finally washed. Examination of this material with the aid of a dissecting microscope revealed a diverse array of megaspores, gymnospermous staminate and ovulate cones, leaves and wood, and angiospermous fruits, seeds and flowers. These are all preserved as three-dimensional compactions, and retain a surprising degree of cellular detail. From this material, which forms the basis of a continuing and more detailed investigation, one particularly well preserved and distinctive flower was isolated, which is described in this preliminary report. It was carefully examined, drawn and photographed, and then embedded in paraffin by standard methods and sectioned at 10 μ m intervals for examination with a compound microscope.

The 3.3-mm long flower (Fig. 1a–c) is apparently bisexual, possibly solitary, and borne on a short pedicel. The five sepals are basally united to form a shallow cup on which the five petals and five stamens are sequentially inserted, the stamens opposite the sepals. The calyx exceeds the corolla, but this may have been caused by the differential erosion of these two floral whorls. The gynaecium is capped by an inflated five-lobed stigma with a central depression. This five-part division is maintained in the cellular structure through the style to the ovary, suggesting the presence of five fused carpels. The compacted nature of the fossil precluded the determination of the number of locules

Fig. 1 a, Photograph of flower in lateral view; note the short pedicel ($\times 12.8$). b Drawing, looking into the flower at a slight angle ($\times 12.8$). c, Interpretative outline; St: stigma (the style below), S: stamen, P: petal, Se: sepal. Note that not all petals are present, and that one sepal is obscured. d, Median section through an umbonate, peltate trichome from the base of a stamen ($\times 269$). e, Tricolporoid/tricolporate pollen (centre) and broken anther sac wall (top left) adherent to the base of one anther (bottom left and right) ($\times 420$).



Dicotyledonous angiosperm flower from the Upper Cretaceous of Martha's Vineyard, Massachusetts

RECENT palaeobotanical studies on Cretaceous angiosperms, including those concerning the antiquity of the conduplicate carpel¹ and the first stages of the evolution of angiosperm pollen and leaf morphology² have contributed significantly to our knowledge of the group. However, the paucity of fossil flowers in the record³ means that existing concepts of the nature of the early flowers of the angiosperms must

and ovules present, their placentation, and the vascularisation of the individual carpels. No stylar canal is present. The laminar anthers lack whole anther sacs, but concentrations of thin-walled debris and pollen suggest that the sacs were embedded in the adaxial face of the stamen, on either side of the connective. The style, the pedicel, and the lower portions of the anthers are ornamented with umbonate, stalked, peltate trichomes which range from 120 to 150 μm in diameter and are up to 20 μm thick (Fig. 1d). Abundant tricolporoidate to tricolporate pollen is found on the adaxial faces of the stamens (Fig. 1e), about the base of the gynaecium, and specifically localised on the five lobes, but not the interlobes, of the stigmatic terminus. The pollen is triangular in polar view, with the apertures borne at the angles. The exine is smooth. The average diameter of the grains is 10–12 μm . The large quantity of pollen adherent to the stigmatic surface implies that the flower was mature at the time of deposition.

The possibility that this flower represents a gymnospermous inflorescence seems remote, particularly in light of the pollen morphology, the presence of a clearly demarcated stigmatic region and the overall spatial relations of the parts. However, although the characteristics of this flower are distinctly angiospermous, their exact association does not seem to occur in any modern group, and attempts to place it in an extant angiosperm taxon have consistently failed. Indeed, the flower seems unique in its juxtaposition of putatively primitive characters (laminar stamens and its firm, resistant texture) with more advanced ones (connate carpels and styles, tricolporoidate/tricolporate pollen, and the small, presumably reduced, number of floral parts). This association of characters is perhaps most notable in that it is advanced beyond those postulated for the most primitive angiosperms^{6,7}, and thus reflects a "minimum" level of evolution achieved by the group by the Campanian. It is tempting to draw parallels between this fossil and various modern taxa. However, to do so would be to force it into the existing preconceived notions of angiosperm evolution, which are primarily based on the living flora.

It is thus deemed more productive to use the approach pioneered by Doyle and Hickey² with respect to leaves and pollen, in which the specimen is compared to other fossil material collected from older and younger horizons, as well as to material from the same horizon. The resulting "relative" taxonomy permits the recognition of trends within the fossil material which might otherwise be obscured. Continued investigation of the floral remains from this particular locality may reveal the presence of a distinctive grade of evolution, serving to elucidate the observed grouping of characters within this fossil as well as to indicate the broader trend of evolution within the dicotyledons at this time in their history.

I thank Mr Clifford Kaye for calling my attention to this locality, and for his interest and support; the selectmen of Gay Head, Massachusetts, for permission to collect at Gay Head cliffs; Professors Carroll Wood and Richard Howard of the Arnold Arboretum of Harvard University for taxonomic expertise, and Professors Elso S. Barghoorn and Rolla Tryon of Harvard University and Dr Leo J. Hickey of the Smithsonian Institution for comments on the manuscript. Figure 1b was drawn by Ms Karen Velmurc, Arnold Arboretum. The research was supported in part by a grant from the Fernald Fund of the Gray Herbarium of Harvard University.

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Effects of plant type, size of geographical range and taxonomic isolation on number of insect species associated with British plants

IN this communication we analyse the insects associated with higher plants other than trees, which have already received attention^{1–5}. We are concerned with two basic problems. First, we ask how various types of higher plants differ in the numbers of species of insects which they support; we do this by comparing standard species–area curves^{1,3,6,7} for five different sorts of plants. Then we consider how the "taxonomic isolation" of a plant, measured simply as the number of related species in the same genus and geographical region, might also influence the total number of insect species to be found on it. We find that while plant type and geographical range have a considerable effect, taxonomic isolation does not, except in the case of monocotyledonous herbs.

Our data come from the accounts of individual plant species given in the *Biological Flora of the British Isles*, published in the *Journal of Ecology* between volumes 36 (1948) and 61 (1973). We excluded the grasses (Graminae) because the insect data appeared to be particularly unreliable. (The entomological literature on which the accounts are based frequently records "grass" as a food plant, rather than the species of grass involved.) An arbitrary series of decisions, inevitably introducing some error, was also made about the accounts which listed no insect species for particular plants. Where an author used a term like "none observed" we included the record as a zero; where he used a term like "no information" we excluded it.

We are aware that many of the *Biological Flora* accounts are incomplete, but since they are compiled by numerous different authors over a long period this seems merely to have increased the variance in the data, rather than introduced systematic errors. It does mean, however, that our statements about the insects associated with different types of plants are based on relative and not absolute comparisons. The present geographical ranges of each of the plants within the British Isles, excluding Ireland (where there are grounds for believing that both the plant distribution and insect data may be less reliable) and the sites of known introductions, were obtained from the *Atlas of British Flora*⁸. The *Atlas* gives the distribution on each species on a presence or absence basis using grid squares of 10 km × 10 km ("10-km squares").

Within the *Biological Flora* data, five distinct groups of plants could be recognised (embracing the families and species listed in Table 1). (1) A group of 27 herbaceous, perennial (or in two cases perennial/biennial) dicotyledons. (The addition of a perennial fern with similar herbaceous growth form, and three creeping perennial but woody dicots made no difference to any of the conclusions listed below; these four species are distinguished in Table 1.) (2) A group of 10 perennial dicotyledonous woody bushes and small shrubs. (3) A group of 16 predominantly annual dicotyledons. Eight of these species are common agricultural weeds (seven annuals and one perennial). The other eight species are all annuals (or in one case annual/biennial) from various natural open or disturbed habitats, and "waste places". (4) A group of 14 monocotyledonous herbs. (5) A small group of four aquatic perennial dicots.

For convenience, these five categories will be referred to as "perennial herbs", "woody shrubs", "weeds and other annuals",

Table 1 Plant families and species in each of five groups recognised in our analyses

(1) <i>Perennial herbs</i> (Species in brackets also assigned to this category are a fern, and three creeping woody dicots.)	
Boraginaceae	<i>Myosotis alpestris</i>
Caryophyllaceae	<i>Silene nutans</i>
Cistaceae	(<i>Helianthemum chamaecistus</i>)
Compositae	<i>Chrysanthemum leucanthemum</i> <i>Cirsium acaulon</i> <i>Senecio jacobaea</i> <i>Succisa pratensis</i> <i>Draba aizoides</i>
Cruciferae	<i>Gentiana verna</i>
Gentianaceae	<i>Teucrium scorodonia</i>
Labiatae	(<i>Thymus</i> spp.) (<i>drucei</i> distribution data used)
Lobeliaceae	<i>Lobelia urens</i>
Papaveraceae	<i>Glaucium flavum</i>
Papilionaceae	<i>Hippocrepis comosa</i> <i>Lathyrus japonicus</i>
Plantaginaceae	<i>Plantago lanceolata</i> <i>P. major</i> <i>P. media</i>
Plumbaginaceae	<i>Limonium vulgare</i>
Polemoniaceae	<i>Polemonium caeruleum</i>
Polygonaceae	<i>Rumex crispus</i> <i>R. obtusifolius</i>
Polypodiaceae	(<i>Dryopteris villarii</i>)
Ranunculaceae	<i>Ranunculus acris</i> <i>R. bulbosus</i> <i>R. repens</i>
Rosaceae	(<i>Dryas octopetala</i>) <i>Sibbaldia procumbens</i> <i>Rubus chamaemorus</i>
Saxifragaceae	<i>Saxifraga hypnoides</i>
Urticaceae	<i>Urtica dioica</i>
(2) <i>Woody shrubs</i>	
Chenopodiaceae	<i>Halimione portulacoides</i>
Elaeagnaceae	<i>Hippophaë rhamnoides</i>
Empetraceae	<i>Empetrum nigrum</i>
Ericaceae	<i>Calluna vulgaris</i> <i>Erica cinerea</i> <i>E. tetralix</i> <i>Phyllocladus caerulea</i> <i>Vaccinium myrtillus</i> <i>V. vitis-idaea</i>
Rosaceae	<i>Potentilla fruticosa</i>
(3) <i>Weeds and other annuals</i>	
Balsaminaceae	<i>Impatiens parviflora</i>
Caryophyllaceae	<i>Corrigiola litoralis</i> <i>Spergula arvensis</i> <i>Chenopodium album</i> <i>Anthemis arvensis</i> <i>A. cotula</i> <i>Sonchus oleraceus/asper</i>
Convolvulaceae	<i>Cuscuta epithymum</i> <i>C. europaea</i>
Cruciferae	<i>Cardaria draba</i> <i>Draba muralis</i> <i>Hornungia petraea</i> <i>Sinapis arvensis</i>
Papaveraceae	<i>Papaver rhoeas</i>
Plantaginaceae	<i>Plantago coronopus</i>
Scrophulariaceae	<i>Melampyrum cristatum</i>
(4) <i>Monocots</i>	
Amaryllidaceae	<i>Narcissus pseudonarcissus</i>
Araceae	<i>Arum maculatum</i>
Cyperaceae	<i>Carex flacca</i> <i>Eleocharis palustris</i> <i>Eriophorum angustifolium</i> <i>E. vaginatum</i> <i>Schoenus nigricans</i> <i>Juncus acutus</i> <i>J. squarrosus</i>
Juncaceae	<i>Allium ursinum</i> <i>Colchicum autumnale</i> <i>Endymion non-scriptus</i> <i>Orchis purpurea</i>
Liliaceae	<i>Sparganium erectum</i>
Orchidaceae	
Sparganiaceae	
(5) <i>Aquatic dicots</i>	
Loleliaceae	<i>Lobelia dortmanna</i>
Menyanthaceae	<i>Menyanthes trifoliata</i>
Nymphaeaceae	<i>Nuphar</i> spp. (<i>lutea</i> distribution data used) <i>Nymphaea alba</i>

“monocots” and “aquatic dicots”. Figure 1 shows their species–area relationships. *S* is the number of species of insect associated with each plant species, and *A* is the geographical range of that plant within the British Isles in 10-km squares. Because the axes are logarithmic, *S*+1 was used so that we could include those species of plants from which no insects were recorded.

The equations are:

Perennial herbs (Fig. 1a)

$$\ln[S+1] = 0.54 \ln A - 0.95 \quad (1)$$

$$r^2 = 0.71; F_{1,29} = 69.57; P < 0.001$$

Woody shrubs (Fig. 1b)

$$\ln[S+1] = 0.45 \ln A + 0.005 \quad (2)$$

$$r^2 = 0.85; F_{1,8} = 45.51; P < 0.001$$

Weeds and other annuals (Fig. 1c)

$$\ln[S+1] = 0.47 \ln A - 1.33 \quad (3)$$

$$r^2 = 0.59; F_{1,14} = 20.32; 0.005 > P > 0.001$$

Monocots (Fig. 1d)

$$\ln[S+1] = 0.39 \ln A - 1.60 \quad (4)$$

$$r^2 = 0.51; F_{1,12} = 12.64; 0.005 > P > 0.001$$

No attempt was made to fit a regression line to the four points available for the aquatic dicots. They are illustrated as Fig. 1a for completeness.

Table 2 summarises a statistical comparison of the four regressions. None of them differs from any of the others in either residual variance or slope; they differ only in intercept. Each of the group of plants has a quite characteristic number of insect species associated with it, for a particular size of geographical range, in the sequence woody shrubs = perennial herbs > weeds and other annuals > monocots. Although the regression line for the woody shrubs lies above that for perennial herbs, the difference is not statistically significant. On the limited data available, the aquatic dicots seem to occupy a position somewhere between the weeds and other annuals, and the monocots.

We next tested the hypothesis⁹⁻¹¹ that taxonomic isolation reduces the number of species of insects associated with a plant, on the grounds that the “exchange” of herbivores between closely related plant species will be easier than “exchange” between unrelated species¹¹. This assumes that, in general, closely related plants are biochemically and structurally more similar than less closely related ones, which does not seem unreasonable^{12,13}. “Exchange” is used here to denote the acquisition of a herbivore guild on an evolutionary timescale. Figure 2 shows the residuals (*R*) derived from the appropriate species–area curves shown in Fig. 1 (equations (1)–(4)), plotted against the number of plant species in the genus (*G*) within the British Isles. (The number of species in the genus on a worldwide basis¹⁴ yielded no sensible patterns.) Log *G* provided the best fits to these data, giving for:

Perennial herbs (Fig. 2a)

$$R = 0.19 \ln G - 0.32 \quad (5)$$

$$r^2 = 0.07; F_{1,29} = 2.07; 0.25 > P > 0.1 \text{ n.s.}$$

Monocots (Fig. 2d)

$$R = 0.30 \ln G - 0.43 \quad (6)$$

$$r^2 = 0.46; F_{1,12} = 10.28; 0.01 > P > 0.005$$

Only equation (6) is statistically significant. There was no relationship between *R* and *G* for either the woody shrubs (Fig. 2b) or the weeds and other annuals (Fig. 2c). The perennial herbs (equation (5)) show signs of the positive relationship predicted by the hypothesis, but this is not statistically significant.

If we view the number of species on a particular plant as a balance between colonisation and extinction, then geographically more widespread plants have a larger number of species associated with them because they are more likely to be found

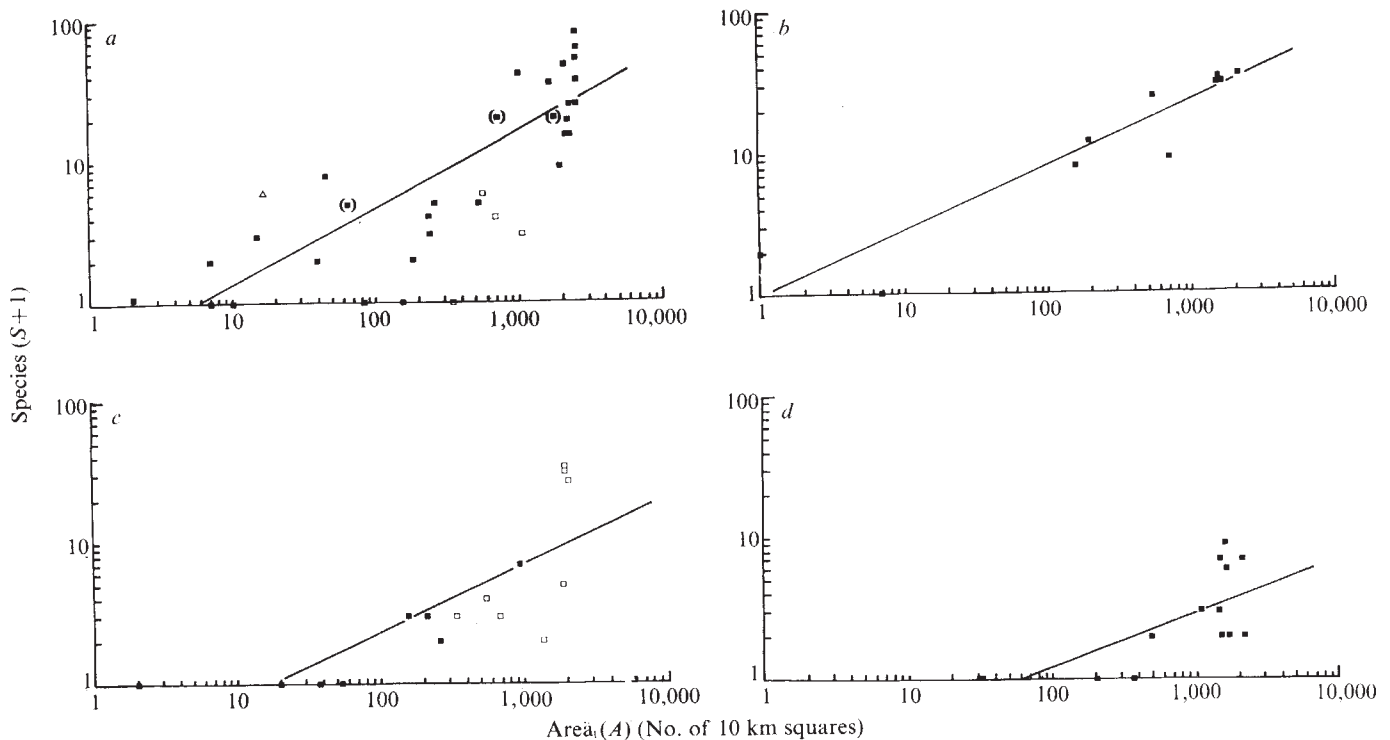


Fig. 1 Insect species associated with various groups of British plants, as a function of the size of the plant's geographical range (measured in terms of the number of 10-km squares occupied by the plant in the *Atlas of the British Flora*⁸). *a*, Perennial herbs. ■, Terrestrial herbaceous dicots; ●, creeping woody dicots; △, perennial fern; □, aquatic perennial dicots (not included in regression line). *b*, Woody shrubs. *c*, Weeds and other annuals. ■, Weeds; □, other annuals. *d*, Monocots (excluding grasses).

and colonised given sufficient time^{1,3,4,9,11}. In Feeny's terminology¹², they are more "apparent". Unpredictability in time and space (weeds and other annuals) reduces "apparency" and increases the probability of extinctions; so does rarity. Hence the equilibrium number of species on rare and/or unpredictable plants is reduced. Why monocots should have even lower equilibria is less obvious. We are inclined to the view that this is a function of their "architecture" with woody shrubs, perennial herbs and monocots forming a series of decreasing structural complexity. Structurally more complex plants permit greater niche diversification (both for competitor avoidance, and the avoidance of your neighbours' predators or parasites^{15,16}) and hence have a bigger equilibrium species pool. Trees are even more structurally complex, and visual inspection of Strong's data for British trees³ suggests higher numbers of associated species, area for area, than woody shrubs or perennial herbs. (A direct comparison is impossible, because

Strong's equation does not fit his own results: also he included, and we excluded, Ireland from the plant-area measurements.) The complete series therefore appears to be trees > woody shrubs = perennial herbs > weeds and other annuals > monocots.

The paucity of insects on aquatic dicots (which fall in this series between weeds and other annuals, and monocots) is well known¹⁷ and presumably reflects low colonisation (or high extinction) rates; why this should be however, is very uncertain.

Monocots excepted, there is no evidence to suggest that taxonomic isolation influences the number of species of insect associated with a particular plant. This may merely reflect the inadequacy of our data, our way of testing for taxonomic isolation, or some fundamental difference between monocots and other plants. For example, the total "pool" of insects adapted to feed on monocots may be so small that it has significantly impaired the probability that a taxonomically

Table 2 Statistical comparison of regressions 1-4

	Woody shrubs (2)	Weeds and other annuals (3)	Monocots (4)
Perennial herbs (1)	$V:F_{29,8} = 2.37$ n.s. $b:F_{1,37} = 0.65$ n.s. $c:F_{1,38} = 2.79$ $0.25 > P > 0.1$ } n.s.	$V:F_{14,29} = 1.01$ n.s. $b:F_{1,43} = 0.34$ n.s. $c:F_{1,44} = 10.41$ } $0.005 > P > 0.001$ }	$V:F_{29,12} = 1.89$ n.s. $b:F_{1,41} = 0.96$ n.s. $c:F_{1,42} = 44.30$ } $P < 0.001$ }
	Woody shrubs (2)	$V:F_{14,8} = 2.39$ n.s. $b:F_{1,22} = 0.02$ n.s. $c:F_{1,23} = 19.20$ } $P < 0.001$ }	$V:F_{12,8} = 1.25$ n.s. $b:F_{1,20} = 0.21$ n.s. $c:F_{1,21} = 72.6$ } $P < 0.001$ }
		Weeds and other annuals (3)	$V:F_{14,12} = 1.91$ n.s. $b:F_{1,26} = 0.23$ n.s. $c:F_{1,27} = 8.51$ } $0.01 > P > 0.005$ }

V, comparison of residual variances; *b*, comparison of slopes; *c*, comparison of intercepts.

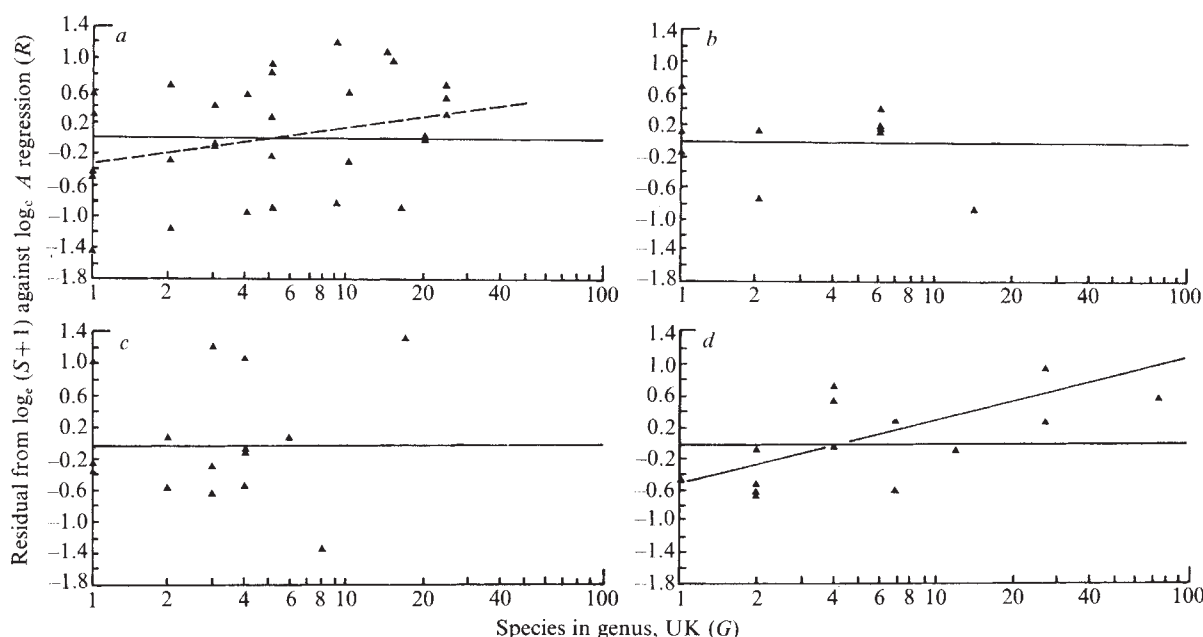


Fig. 2 The residuals from the fitted regression lines in Fig. 1, plotted against the number of species of plants within each genus in the United Kingdom. (The number of plant species in each genus is a measure of "taxonomic isolation".) *a*, Perennial herbs; *b*, woody shrubs; *c*, weeds and other annuals; *d*, monocots. Only the monocots (*d*) show a significant relationship.

isolated member of this group will be colonised over evolutionary time. With a larger total "pool" (like that on perennial herbs) the effects of taxonomic isolation have been lost. This viewpoint obviously demands that the insects which exploit dicots have a negligible probability of contributing to the monocot "pool". It also raises some important questions about what is meant by an equilibrium assemblage³⁻⁵, and the time scale over which this should be measured. These problems are beyond the scope of the present note.

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Evidence, using radioactive phosphorus, of interspecific food exchange in ants

MEMBERS of many ant species exchange fluid foods within the colony¹⁻⁴. Similar exchange between social symbionts, living as "slaves"⁵ or "guests"⁶ and their hosts, is also documented¹. However, in non-symbiotic species, found in the same habitat or in distinctly different habitats, this phenomenon is usually overlooked. We document here that the food-transporting worker ant of one species offered carbohydrate food to the aggressive worker ants of another as a means of suppressing aggression in the latter, as in appeasement displays in higher animals. The workers of the aggressive red fire ant species, *Solenopsis invicta*, introduced into the southern United States in the 1930s but comparatively recently named⁷, were invariably offered regurgitated fluids by indigenous species *S. geminata* and *Pheidole dentata* which had been fed earlier with ³²P-labelled 20% sucrose solution.

In 1.5 cm diameter, 1.5 cm wide glass cells, where the donors and aggressors could encounter each other quite often, a single donor worker offered the regurgitate to four aggressors within 15 s of their first encounter, and all the aggressors were offered food within 20-30 min. Such a food offer was made regardless of the aggressor's caste. Both the *Solenopsis* species are polymorphic and *P. dentata* is dimorphic. In addition to major and minor castes, the polymorphic species contained a gradation of medium-sized workers. Immediately following antennal contact between the donor species and the aggressor species, both opened their mandibles: the aggressor apparently to alarm its sister workers and to seize the body parts of the donor, whereas the donor acted thus to regurgitate a large drop of fluid between its mandibles. As the alien species became more aggressive, seizing the donor, stinging, and so on, the food-offering responses of the donor also intensified: the donor contacted the aggressor's antennal bases with its vibrating antennae and oriented the food droplet towards the aggressor (Fig. 1). When the donor was kept with workers of its own species, the workers contacted its antennal bases with theirs, in food solicitation. In some cases, food was offered only after the solicitors groomed the donor with their mouth parts in a ritualised fashion from legs up to the cephalic ventrum. The contrast in food-offering behaviour by one donor worker towards its own sister workers, and the aggressive workers of an alien species, suggested

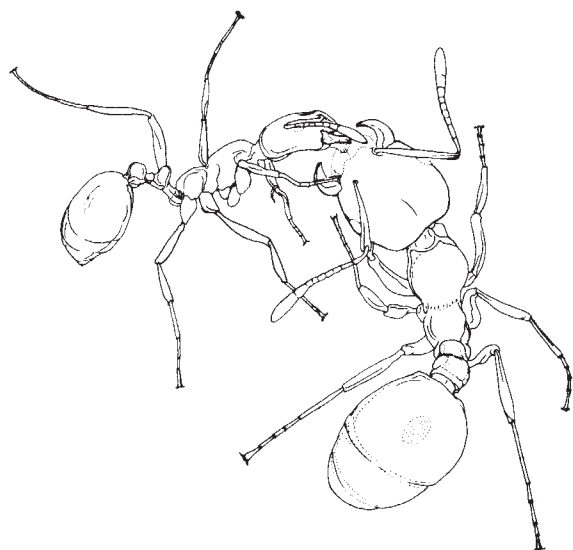


Fig. 1 Unusual interspecific trophallaxis between a donor of *Solenopsis invicta* (major worker with hatched spot on abdomen) and an aggressive solicitor minor worker of *S. geminata* drawn from actual photograph. The "appeased" solicitor touched the donor's mandibles with its forelegs and held antennae near head while feeding. The donor held its antennae in a W-shape and moved them to and fro while being solicited. The donors are fed on $^3\text{H}^{32}\text{PO}_4$ (specific activity 1.8 mCi ml^{-1}) in 150 g Gerber baby food jars for 2 h. They were made less mobile by exposure to CO_2 for 5–10 s and were surface decontaminated by passing through inactive NaH_2PO_4 solution¹. The radioactivity was measured by using a GM-end-window (thickness $1.2\text{--}1.4 \text{ mg cm}^{-2}$) counting tube in conjunction with a Berthold Scaler Timer BF 22/25.

that in the latter case, the readiness of the donor to offer food reduced the aggression in the aggressor species.

In the southern United States, where *S. invicta* is regarded as a nuisance, it subdued under laboratory conditions the colonies of over 30 species, comprising six subfamilies. It killed twice its number of *S. geminata* and four times its number of *P. dentata* when their workers confronted each other in large numbers⁸. When the workers of *S. geminata* and *P. dentata* were presented to each other in the ratio of four aggressors to one donor, and *S. invicta* was a donor species, food transfer occurred. No sooner had the donor offered its alimentary fluid to the aggressors, than their aggressive frenzy diminished and the food was accepted. The food was aggressively solicited a number of times within the short

period of 30 min. The workers that did not accept food were especially aggressive, that is they elicited mandibular attack, stinging, and so on. The alien workers accepted food and groomed themselves. In the laboratory, the donor showed escape behaviour by crawling on the glass cell. In nature, the aggressor's pause to eat and groom itself could make the donor's escape possible.

Variations in the distribution of food from major or minor caste donors to similar or dissimilar castes of aggressive solicitors, in all the three species, may be due to the differential aggressive element present in individuals or castes, and the individual's own food reserve. This explanation may hold true for less frequent food transfer from the workers of *S. invicta* to the workers of *S. geminata* or *P. dentata*, that is the aggressive element in *S. invicta* dominated that in the other two species (Table 1). An overt form of defensive behaviour in the major workers of *S. geminata* and *P. dentata* with the use of a novel alarm-recruitment system has been documented⁹. During the colony confrontation studies between *S. invicta* and two species of *Pheidole*, the guarding workers of *P. dentata* and *P. morrisi* exchanged food with the nest-invading *S. invicta* workers and delayed their invasion by 20–30 min (A. P. Bhatkar, unpublished).

Monomorphic species where workers exchange fluid food within the colony, such as a species of subgenus *Diplophoptrum*, *S. pergandei*, and *Monomorium pharaonis*, escaped the attacks of *S. invicta* by slipping through their wide open mandibles. The workers of both these species are approximately half the size of the smallest *S. invicta* (smallest *S. invicta* 3.2 mm, largest 7.5 mm). During prolonged exposures (20 min–60 min), however, *S. invicta* obtained food by piercing the soft cuticle of slow moving, less aggressive donors. Similarly, otherwise aggressive woodland species with monomorphic workers (1.5 times as large as the largest *S. invicta* worker), *Aphaenogaster lamellidens*, fell victim to *S. invicta* due to diminished aggression and mobility in the donors. We may add that intracolony exchange of fluid foods was absent in *A. lamellidens*.

Exchange of alimentary liquids (solutions, emulsions and suspensions) between colony members, including social symbionts, is usually termed "trophallaxis"⁹. If the scope of this definition is appropriately widened, the occurrence of food transfer in nonsymbiotic species of the type described above could very well be termed "interspecific trophallaxis". Interspecific associations in the ant colonies have one common denominator which is that ultimately they are all trophic in relation. The development of all forms of social symbioses among ant colonies, from plesiobiosis (nesting in proximity with little or no communication), xenobiosis (food sharing, keeping separate broods), to dulosis (one species robbing the brood of another and increasing its common worker force) and inquilinism (one species passing its

Table 1 Food exchange in different ant species expressed in terms of transfer of radioactivity from donor to recipient workers

Donor species and caste	Recipient species and caste	Radioactive ants per ants exposed in all replicates	c.p.m. radioactivity per recipient	
			mean	s.d.
<i>S. geminata</i>	<i>S. invicta</i>			
minor	minor	16/16	62.88	136.50
minor	major	16/16	21.00	16.13
major	minor	8/16	9.69	20.77
major	major	14/16	254.75	589.49
<i>P. dentata</i>				
minor	minor	11/16	105.75	169.61
minor	major	6/12	301.16	547.49
major	minor	13/16	310.19	466.76
major	major	4/12	175.00	434.63
<i>S. invicta</i>	<i>S. geminata</i>			
minor	minor	9/16	45.63	169.61
<i>S. invicta</i>	<i>P. dentata</i>			
minor	major	5/16	7.75	9.37

Food distribution from donor to recipients, and secondarily, among recipients was not normal. High s.d. was due to some workers receiving food repeatedly while the others in small amount. Experiments were conducted at 28 °C, replicated three or four times, and the exposure of one donor to four aggressive solicitors in each replicate was 1–5 h.

life cycle at the cost of other) seem to have interspecific food exchange as a driving force in their evolution. Such a phenomenon among phylogenetically and ecologically separate species is possibly an adjustment against competition even when they come together by accident, such as the red imported fire ant from Brazil and the Florida Myrmicinae, described here.

This research was conducted at the University of Florida, Gainesville, in 1972 when the authors were graduate student and visiting professor respectively.

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Presumptive evidence of two active X chromosomes in somatic cells of a human female

In a female child with various morphological abnormalities and partial translocation trisomy X—karyotype: 46,XX,—22,+der(22),t(X;22)(q12;p11)mat—we have found evidence of two euchromatic X chromosomes in about 15% of metaphases from lymphocyte cultures. We believe this means that in these cells there were two active X chromosomes.

After treatment with BUdR (200 $\mu\text{g ml}^{-1}$) during the final 7 h of culture different patterns of decondensation were observed in individual metaphases. In 50% of cells an entire X and the long arm of the X/22 translocation chromosome, der(22), were decondensed (Fig. 1). In a smaller proportion of mitoses, however, the two normal Xs were of equal length, showing a reversed banding pattern, whereas the long arm of der(22) was highly decondensed (Fig. 2). In another cell line the der(22) and one whole X showed

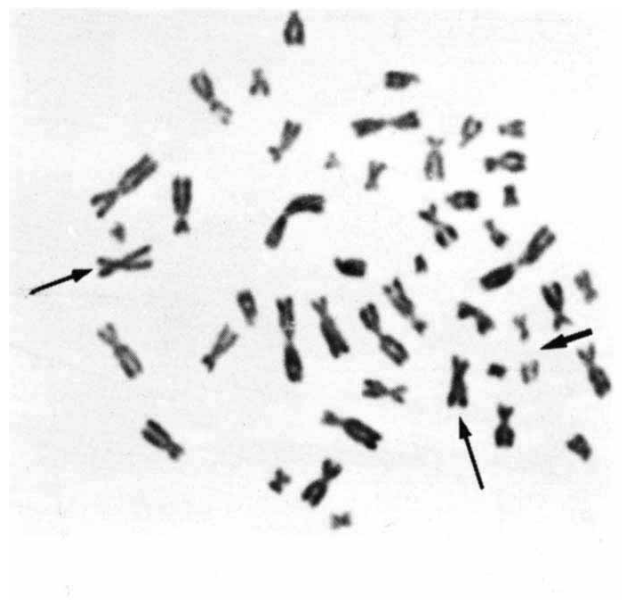


Fig. 2 Type 1 cell. The thin arrow shows euchromatic Xs, and the thick arrow shows heterochromatic long arm of der(22) (verified by Q fluorescence).

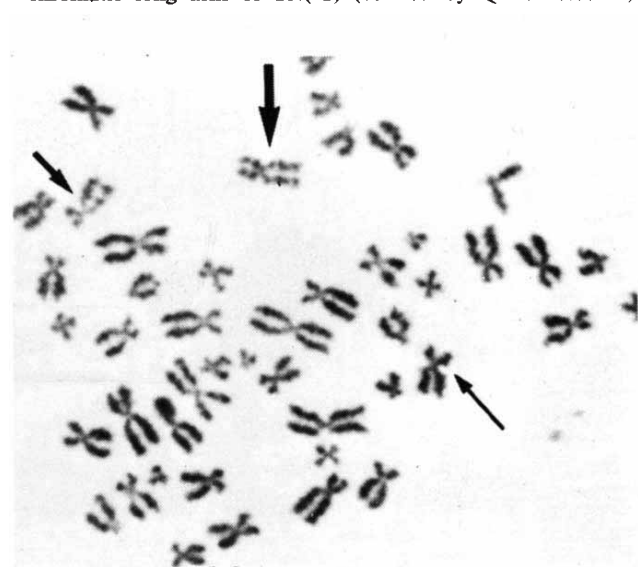
normal condensation, the other X being decondensed (Fig. 3). The rest of the karyotypes were uninformative.

After treatment of blood cultures with ^3H -thymidine ($1 \mu\text{Ci ml}^{-1}$) for the final 7 h of culture three different late replication patterns were observed. (1) In the majority of suitable metaphases one entire X and the der(22) were heavily labelled; (2) in a smaller proportion of cells only the long arm of the der(22) was late labelling, the two normal Xs showing only a few grains. (3) In a few mitoses one X was late replicating, whereas the other X and the der(22) showed an early pattern.



Fig. 3 Type 2 cell. The thin arrow shows euchromatic X; the medium arrow, euchromatic der(22), and the thick arrow, heterochromatic X (verified by Q fluorescence).

Fig. 1 Type 3 cell. The thin arrow shows euchromatic X; the thick arrow, heterochromatic X, and the medium arrow, heterochromatic long arm of der(22) (verified by Q fluorescence).



Percentages and sample sizes are summarised in Table 1. The different percentages of type 2 and type 3 cells found with BUdR and autoradiography respectively can probably be explained by the small sample size. (The uninformative observations are not entered in Table 1. They account for the differences between summed percentages and 100%.)

Four different sex chromatin patterns were found in buccal mucosa cells, hair root cells and fibroblasts (Fig. 4).

Table 1 Patterns of labelling

Cell type	Blood cultures		Observations		Sex chromatin		Interpretation
	Decondensation BUdR	Late labelling ³ H-thymidine	Hair roots		Buccal mucosa	Fibroblasts*	
1	der(22) 12.5%	der(22) 17%	S 14%		S 10%	S 16%	XX
2	X 25%	X 7%	N 26%		N 35%	N 32%	XXq
3	X der(22) 50%	X der(22) 76%	N+S 4%		N+S 11%	N+S 10%	X
4	XX 0%	XX 0%	2N 4%		2N 16%	2N 10%	(Xq)‡
Total cells examined	32	42	250		500	600	

N, normal sized Barr body; S, small Barr body.

* Courtesy of Dr W. Vogel, Institute of Human Genetics; Freiburg im Breisgau.

† Xq, Xq12 → Xqter.

‡ See text.

There were single normal sized Barr bodies, double Barr bodies of equal size, double bodies of different size and single small bodies. The latter are thought to be derived from the der(22) chromosome, consisting of Xq only, and the normal sized sex chromatin bodies are thought to be derived from the morphologically normal X. Two equal sized Barr bodies would then suggest the presence of cells with two equal sized heterochromatic Xs. This type of cell (type 4, Table 1), however, was never identified in blood cultures with either BUdR or ³H-thymidine. The nuclei with two Barr bodies of apparently equal size were probably cells with one entire X and the der(22) inactivated, the difference in size between the two not being detectable in every interphase nucleus. The size of the Barr bodies in cases of Xq- or i(Xp) as well as Xp- has been reported sometimes to appear normal, for example as reported by Van den Berghe *et al.*¹ and Atkins *et al.*². The most striking discrepancy between metaphases of cultured blood cells and sex chromatin findings in interphase nuclei was the relative paucity of nuclei with double bodies, especially in hair root cells and fibroblasts, compared with 50% and 76% of blood cells with one X and the der(22) inactivated (type 3, Table 1). It is well known, however, that in proven cases of triple X syndrome only a certain percentage of cells shows double Barr bodies, just as in normal females only between 20% and 50% of interphase nuclei are sex chromatin positive, varying according to the tissue examined and the method used. There is very good agreement with respect to cells with two active Xs (type 1, Table 1) and fair agreement for

cells of types 1 and 2 taken together, allowing for the fact that some of the cells with one normal sized body are probably cells of type 3 with one X and the der(22) inactivated. It is also possible that the four cell types are present in different ratios in different tissues. Type 4 cells (Table 1), probably do not exist in lymphocytes. Whether they exist in other tissues or whether the recorded double Barr bodies of equal, normal size were Barr bodies derived from structurally different chromosomes (X and der(22)) corresponding to type 3 cells remains to be shown.

The mother of the patient had normal sex chromatin counts. She had the same translocation chromosome der(22) in addition to one normal and one deleted X, consisting of Xp, centromere and proximal Xq—karyotype: 46,X,t(X;22)(q12;p11). She had a normal phenotype.

At 6 months the child had failed to grow and gain weight and had deformities of head and face with low set ears, small lower jaw, upward slant to the eyes as in mongoloids, beaked nose, flat bridge of the nose, epicanthal folds, congenital heart disease and hypertonus of muscles. This phenotype cannot be explained solely by the partial trisomy X. It could be explained by monosomy 22 through spread of inactivation on the translocated 22. But the translocated 22 never showed any elongation or fuzziness or decondensation indicating inactivation (Figs 1 and 2) as shown for two X/autosome translocations by the BUdR method³ and autoradiographically for an X/21 translocation⁴. In sufficiently stained metaphases with straight (neither bent nor twisted) chromosomes no label was seen over the short arm of the der(22) chromosome (Fig. 5) in neither cell type. If this should prove to be the case in other tissues, the reason for the malformations of the child must be sought in the gross genetic unbalance caused by noninactivation of

Fig. 4 Barr bodies in fibroblasts. a, Type 1, small Barr body; b, type 2, normal Barr body; c, type 3, small plus normal Barr bodies; d, type 4, two normal Barr bodies.

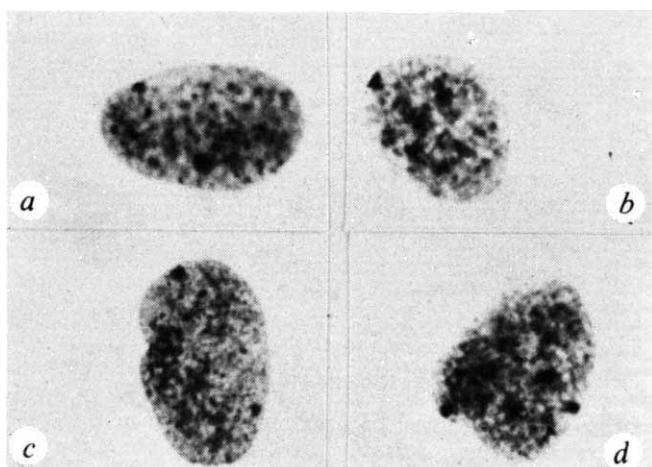
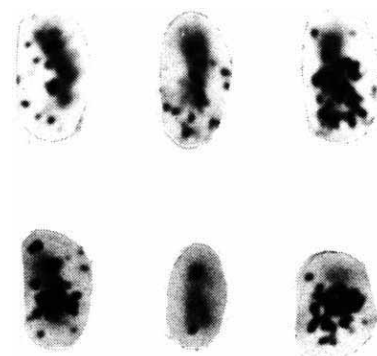


Fig. 5 X chromosomes and der(22) (verified by trypsin Giemsa banding) from two metaphases (type 1 upper, and type 3 lower) labelled with ³H-thymidine. Note unlabelled short arm of der(22).



all X material in excess of one whole X, provided that we can equate decondensation and late replication with genetic inactivity.

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¹ Van den Berghe, H., Fryns, J. P., and Devos, F., *Humangenetik*, **20**, 163–166 (1973).

² Atkins, L., Santesson, B., and Voss, H., *Ann. hum. Genet.*, **29**, 89–95 (1965)

³ Dutrillaux, B., *Chromosomes Today*, **5**, 395–407 (1974).

⁴ Summit, R. L., Martens, P. R., and Wilroy, R. S., *J. Pediatr.*, **84**, 539–546 (1974).

Pattern formation in the absence of polarity in *Dictyostelium discoideum*

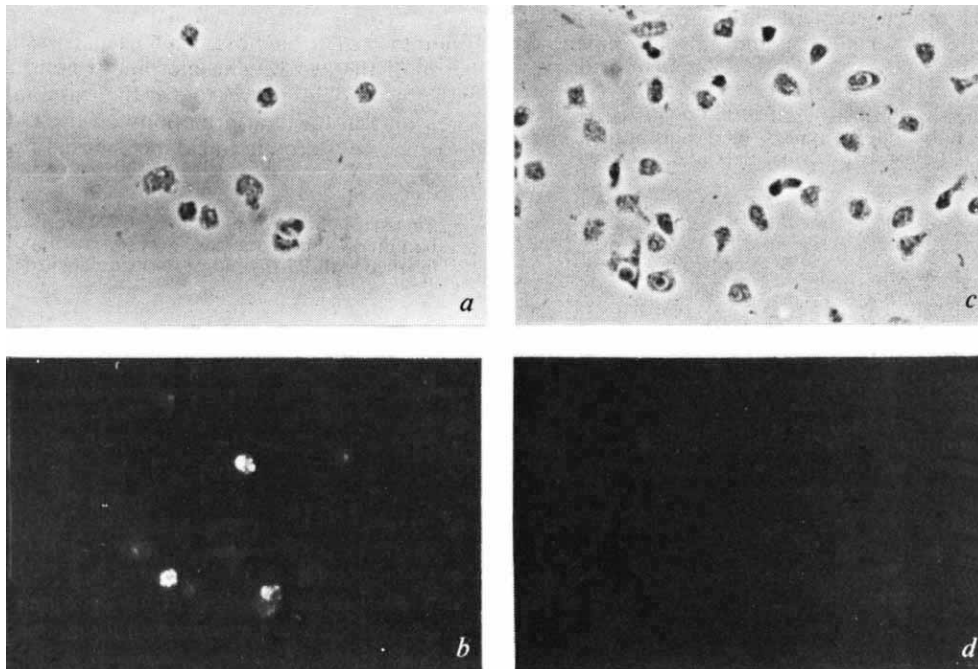
MANY theories have been advanced as to how the spore-stalk pattern of the slime mould *Dictyostelium discoideum* is established. Without exception these envisage the polarity of the grex stage of the life cycle as a key factor in specifying the pattern. One group of theories^{1–4} sees the combined influence of polarity and the grex tip as signalling positional information^{5,6} which determines that the front cells differentiate into prestalk cells and the back cells into prespore cells. An alternative view is that pattern formation occurs by sorting out within the grex of cell types which are pre-

determined at an earlier stage of the life cycle^{7–11}. Grex polarity is also a key factor in one suggested mechanism for sorting-out in that prestalk cells are seen as moving faster than prespore cells and therefore coming to the front of the migrating grex^{8,12}. Because all these theories clearly suggest that pattern formation ought not to occur in circumstances in which the cell mass is unpolarised, we asked the question: 'Is polarity necessary for differentiation and pattern formation in *D. discoideum*?' We have found that a prestalk-prespore pattern very similar to that seen in the normal life cycle forms in spherical aggregates which, before pattern formation, have no detectable morphological polarity. We also describe the formation of unusual cyst-like structures from patterned spherical aggregates.

Spherical aggregates were formed by allowing axenically grown log phase cells of *D. discoideum* (strain AX-2) to adhere together in rotated suspension in phosphate buffer¹³. In such conditions cells begin adhering immediately, eventually forming spherical aggregates of about 100 μm in diameter. The cells became aggregation competent after approximately 8 h (ref. 14).

The first question we asked was whether differentiation of prespore cells would take place in such aggregates. This was tested by collecting aggregates at different times, dissociating them into single cells which were allowed to stick to a slide, and fixing and staining these with a fluorescein-labelled anti-spore serum prepared against the spores of *D. mucoroides* (modified from the method of Takeuchi), ref. 15 and D.F., and D.R.G., in preparation), (Fig. 1a and b). We found that specifically staining intracellular vesicles characteristic of prespore cells began to appear in a proportion of the cells between 12 and 13 h of shaking. By 17–18 h the vesicles stained more intensely and

Fig. 1 $\times 1,000$. Cells dissociated from 18-h aggregates observed under phase-contrast (a) and under ultraviolet light (b), having been stained with specific anti-spore serum labelled with fluorescein isothiocyanate (FITC). Some of the cells show the development of specific prespore vesicles (PSVs). The same cells shaken more vigorously (c) remain single and on staining (d) show no specific reaction. *Dictyostelium discoideum* vegetative cells (strain Ax-2) were harvested from axenic growth medium¹⁸, washed three times in cold distilled water, and resuspended in 0.0167 M phosphate buffer (pH 6.0) at a density of 10^6 cells ml^{-1} . Aliquots (4 ml) of the cell suspension were put into 25-ml flasks on a rotary shaker at 140 r.p.m. (radius of rotation $\frac{1}{4}$ inch; temperature 22 °C). The aggregates formed at 18 h were dissociated into single cells by trituration in cold distilled water. The single cells were suspended in standard salt solution¹⁹ at 2×10^5 cells ml^{-1} and allowed to settle over and adhere to glass coverslips. Fixation and staining of cells with specific antispore serum was carried out as previously described¹⁵. For fluorescent microscopy cells were observed on a Zeiss Universal microscope, fitted with an HBO 200 mercury arc lamp, BG 38 and BG 12 exciter filters and Zeiss barrier filters 50 and 44. Cells treated with a control non-immune serum showed no specific staining. Cells were kept single and prevented from forming aggregates by shaking at 260 r.p.m. These cells were tested for aggregation competence at 8 h by allowing them to settle on to coverslips and looking for the formation of characteristic elongated shapes and development of end-to-end chain-like contacts between cells.



were present in a greater proportion of the cells. These vesicles seemed identical to the prespore vesicles (PSV) which have been identified in late aggregates and grexes when cells are allowed to develop in normal conditions on a surface (refs 15, 16 and D.F., and D.R.G., in preparation). The time of appearance of prespore cells in spherical aggregates was approximately the same (that is, 12 h after the end of the vegetative phase and 4 h after chemotactic aggregation) as in cells undergoing normal development (D.F., and D.R.G., in preparation).

When vegetative cells were kept single in suspension by vigorous shaking, they became aggregation competent after about 8 h even though they did not form aggregates. Would such cells also differentiate into prespore cells? Even after 36 h of shaking in suspension single cells showed no sign of staining with specific anti-spore serum though they remained viable (Fig. 1c and d). It seems that single cells are capable of differentiation to the aggregation stage but cell contact is an essential prerequisite for further differentiation into prespore cells.

Since morphological polarity of aggregates was not essential for prespore differentiation, we proceeded to ask whether the formation of a prespore : prestalk pattern could also occur in the absence of polarity. Fluorescent antibody staining of sections through 18 h aggregates showed no obvious patterning of the arrangement of stained and unstained cells (Fig. 2a). By 24 h, however, there was a clear spatial separation of the two cell types (Fig. 2b). Examination of many randomly sectioned aggregates showed the structure to be a mass of stained prespore cells partially surrounded by a cap of unstained cells. When 24-h aggregates were plated on to an agar surface they developed directly into normal fruiting bodies.

Because differentiation and pattern formation could proceed to a considerable extent within spherical aggregates by 24 h, shaking was continued for longer periods to see whether differentiation into mature spores and stalk would occur. We found that after 4 d of shaking, aggregates formed cyst-like structures unlike any morphological arrangement previously reported. These consisted of three concentric layers, an outer non-cellular coat, a layer of loosely packed cells and a central core of tightly adhering cells (Fig. 3a). Both the outer coat and the surfaces of the outer layer of cells exhibited specific staining with antispore serum. Cells in the central core were not stained (Fig. 3b). On the basis of phase contrast microscopy we concluded

Fig. 2 Sections through 18 h (a) and 24 h (b) aggregates stained with FITC-labelled anti-spore serum and observed under ultraviolet light. Aggregates were settled out from phosphate buffer, fixed in 95% ethanol, dehydrated, wax embedded, and sectioned at 5 μ m according to the method of Sainte-Marie²⁰. Staining was the same as for single cells. a, $\times 1,250$; b, $\times 625$.

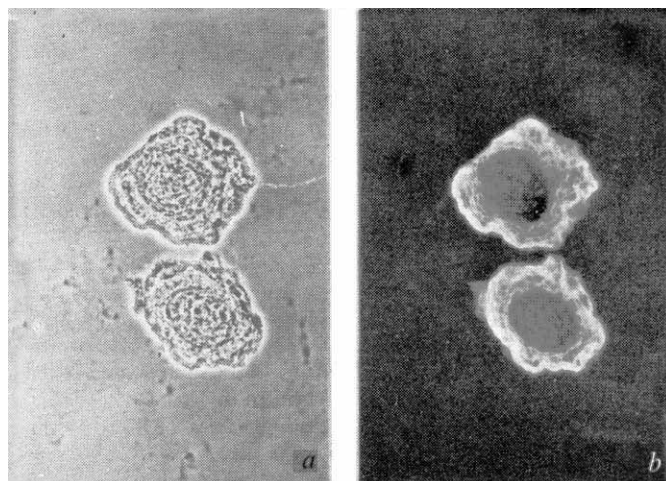
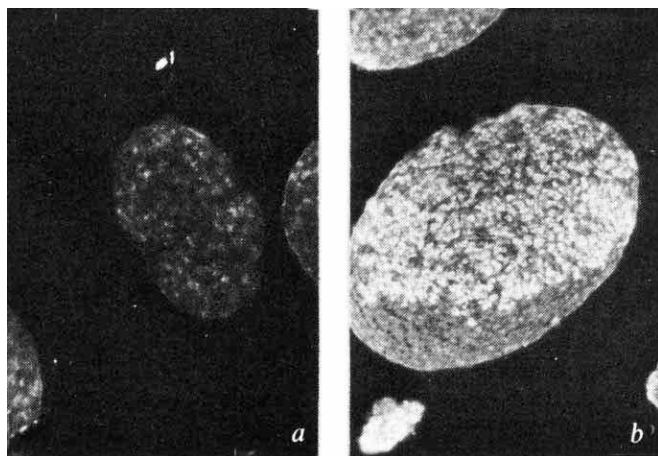


Fig. 3 Sections through 96-h aggregates under phase contrast (a) and ultraviolet light (b) having been stained with FITC-labelled anti-spore serum $\times 625$.

that neither of the two cell types in the cysts structurally resembled either the spores or the stalk cells of normal fruiting bodies. Examination of stages intermediate between 24 and 96 h revealed a progressive accumulation of fluorescent-staining material at the aggregate surface and a corresponding decrease in the number and intensity of staining of PSVs. We suggest that although mature spores were not seen in these cysts, the vesicles in the prespore cells release their contents to the cell surface in the same way as do prespore cells during normal culmination^{16,17}, and that this material moves to the outside where it forms the coat of the cyst. Cyst formation is being further examined by electron microscopy. 4-d cysts did not develop when simply plated onto an agar surface but gave normal fruiting bodies within 4–5 d when plated with a bacterial food source.

The most important conclusion from these results is that differentiation and pattern formation, ostensibly similar to that found in the normal life cycle, can occur amongst *D. discoideum* cells in conditions where anterior-posterior polarity and grex tip formation are absent. The only necessary conditions for prespore differentiation which we can identify at present are (1) that the cells should be 12 h post-feeding and (2) that they should be in mutual contact. PSV formation may be added to a number of differentiative processes mediated by cell contact following aggregation^{18,19}.

Since there is no obvious spatial organisation in the early appearance of prespore cells, we suggest, in common with Takeuchi¹⁵, that the cell types differentiate at random within the aggregate and then give rise to the pattern by sorting-out. (That spatially random differentiation can occur within populations of slime mould cells is shown by the random establishment of aggregation centres within a uniform field of amoebae²⁰. Proof that prespore and prestalk cells can also differentiate at random requires further experimentation.) Although our observations do not provide definitive proof of sorting-out, they are consistent with such an interpretation, and there is good evidence that sorting-out of slime mould cells does occur^{7–11}. In addition it is well known from extensive studies on vertebrate embryo cells that different cell types adopt the types of configuration which we have found by sorting-out within aggregates^{21,22}. Our results are inconsistent with differentiation in position because we have never observed in 18-h aggregates in a region devoid of prespore cells comparable in size to the non-staining regions of 24-h aggregates.

We propose the following new view of pattern formation in the slime mould. It has been shown that during the normal life cycle, differentiation of cell types slightly precedes

the formation of a grex tip²³, and so it may be suggested that pattern formation occurs by sorting-out which begins before tip formation and that it is the early prestalk region which gives rise to the grex tip. Viewed in this way, it is the prestalk-prespore pattern which generates the polarity of the cell mass rather than the polarity which generates the pattern. Pattern formation certainly precedes the migrating grex stage²⁴ but it remains to be determined at precisely what stage during the normal life cycle the spatial pattern actually begins to form. Such a view leaves unexplained the mechanism governing the numerical proportions of mature spore and stalk cells which are known to be present in approximately the same proportionate ratio irrespective of the size of the cell mass²⁵⁻²⁷. We suggest (and we know of no evidence which contradicts this view) that the method of proportioning is independent of the mechanism which determines the spatial arrangement of cell types and the polarity of the cell mass.

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seem to suggest an association between this chromosome abnormality and defective chemotaxis.

Patients 1 and 2 had blood and bone marrow findings indicating preleukaemia³; acute leukaemia later developed in patient 2. Patient 3 had acute myeloid leukaemia known to have been preceded by a preleukaemic phase. Patient 4 had acute myeloid leukaemia when first seen, and patient 5 had subacute myelomonocytic leukaemia. Patients 1 and 3 had distinctly increased susceptibility to bacterial infections, mainly pneumonias and abscesses. Patient 2 also suffered from repeated febrile episodes probably of infectious origin, but no conclusive evidence of infections could be obtained. Since defects of leukotactic function occur in chronic and acute bacterial infections^{4,5}, our tests were performed at least 1 week after the disappearance of any sign of infection. Patient 4 had been treated with cytostatic agents before testing, but not within 10 d before the experiments.

Chemotaxis was tested in patients 1, 2, 3 and 4 by a modification of the Boyden method⁶ and in patient 5 by another modification⁷ of the same. In the former method the results are expressed as the average number of cells per high power field that have migrated through a Millipore filter in 3 h, in the latter as the distance which the leading front of cells has migrated within the membrane in 1 h.

Table 1 Chemotaxis of neutrophils

Patient	Age (yr)	Sex	Cells	Chemotactic attractant	Cells per field
1	49	M	NC	NS 56° 30 min	0.2 ± 0.2*
			NC	NS zy	65.0 ± 3.0
			PC	NS zy	16.0 ± 1.3
			NC	PS zy	112.7 ± 4.9
2	49	M	NC	NS 56° 30 min	0.9 ± 0.5
			NC	NS zy	71.2 ± 4.2
			PC	NS zy	6.1 ± 0.5
			NC	PS zy	87.4 ± 4.8
3	29	F	NC	NS 56° 30 min	3.0 ± 0.6
			NC	NS zy	52.4 ± 4.1
			PC	NS zy	8.7 ± 1.4
			NC	PS zy	55.7 ± 2.9
4	56	F	NC	NS 56° 30 min	0 ± 0
			NC	NS zy	25.6 ± 2.1
			PC	NS zy	10.4 ± 1.0
			NC	PS zy	23.1 ± 1.5
5	68	F			Distance migrated (µm)
			NC	Hanks	19.6 ± 0.9†
			PC	Hanks	17.2 ± 0.5
			NC	Casein	87.4 ± 3.9
			PC	Casein	70.5 ± 2.1
			NC	NS 56° 30 min	51.5 ± 1.8
			NC	PS 56° 30 min	48.1 ± 1.5
			NC	NS zy	94.7 ± 2.1
			NC	PS zy	89.3 ± 4.1

Assay conditions: patients 1, 2, 3 and 4, cell concentration was adjusted to 2×10^6 neutrophils ml^{-1} , and 10% pooled human serum was used in the cell suspension throughout. Modified Boyden chambers⁶ were set up in triplicate. 30% heat-inactivated (30 min at 56 °C) or zymosan-activated (30 min at 37 °C) serum was used in the lower compartment, as indicated in the table. Hanks' solution was used as the medium. The cells were allowed to migrate for 3 h at 37 °C. Results are expressed as the average number of neutrophils per high power field ($\times 400$) on lower surface of Millipore filters (3-µm pore size) separating the upper compartment of the chamber from the lower one. Cells were counted in ten high power fields on each filter. Patient 5, cell concentration was adjusted to 1×10^6 neutrophils ml^{-1} . No serum was used in the cell suspension. 10% serum was added to the attractant compartment as indicated. The cells were allowed to migrate into the filters for 55 min towards casein (5 mg ml^{-1}) or serum, and for 90 min towards Hanks' solution, after which the leading front⁷ of the cells was determined in five fields of each of six filters. The mean $\pm$ 2 s.e. of our normal material (40 individuals) for migration towards Hanks' solution is 20.7 ± 3.7 µm, and towards casein 99 ± 18 µm.

*Mean $\pm$ 1 s.e. of ten high power fields in each of three filters.

†Mean $\pm$ 1 s.e. of five determinations in each of six filters.

NC, Normal cells; PC, patient cells; NS, pooled normal serum; PS, patient serum; zy, zymosan.

Defective chemotaxis in monosomy-7

CELLULAR defects of chemotaxis are rare disorders¹. Little is known about the cellular mechanisms of the chemotactic response, and in most cases the nature of the defect remains obscure. Singh *et al.* have reported defective chemotaxis in association with a chromosomal abnormality². In their patient, bone marrow cells were tetraploid. In most cells a chromosome 16 was missing and there was an extra C-group chromosome. We investigated neutrophil granulocyte function in haematological disorders. Our results from a study of neutrophil function in five patients with monosomy-7

Table 2 Bone marrow chromosome findings

Patient	Time	Chromosome counts						Total	Most prevalent karyotype
		≤43	44	45	46	47	≥69		
1	XII 1972	1	2	18	—	—	—	21	45, XY, -7
	IX 1973	—	—	1	—	—	—	1	
	XI 1973	—	1	11	—	—	4	16	
	I 1974	—	5	11	—	—	1	17	
	III 1974	—	—	5	1	—	1	7	
	IX 1974	—	2	8	—	—	1	11	
2	X 1973	—	—	5*	21†	—	4	30	46, XY 45, XY, -7, del (20) (q11)
	IX 1974	—	—	21	—	1	2	24	
	IV 1975	—	—	12	—	—	—	12	
	IX 1973	—	—	11	9†	—	1	21	
3	X 1973	1	—	12	6†	—	1	20	45, XX, -7
	VIII 1974	—	—	15	2	—	—	17	
	IX 1974	2	1	10	1†	—	1	15	
	V 1975	—	—	9	2†	—	—	11	
	VIII 1974	—	—	13	—	—	—	13	
4	IX 1974	—	—	19	1	—	—	20	45, XX, -7 (Ph ⁺)
	III 1974	—	1	14	—	—	2	17	
5	II 1975	—	—	21	—	—	—	21	45, XX, -7
	III 1975	—	—	10	—	—	—	10	

* Two cells were 45, XY, -C.

† Normal karyotype.

In all patients the karyotypes of leukoagglutinin-stimulated peripheral lymphocytes were normal.

Casein is used as a chemoattractant in the latter modification in addition to serum. The concentration of mature neutrophils (band and segmented) was constant in all experiments.

Our method for bone marrow chromosome work has been described in detail previously⁸. The routine method for banding practised in all cases was Giemsa staining of trypsin-treated slides. Q- and C-banding was performed in selected cases.

The response of cells from patients 1, 2, 3 and 4 to zymosan-activated serum was clearly inferior to that of control cells (Table 1). Good chemotactic activity could be developed with patient sera. These results indicate a cell-associated defect of chemotaxis. Owing to a switch to a new method which also allows the measurement of the random migration of cells, the results of patient 5 are not quantitatively comparable with the others. A possibly reduced chemotactic response was seen in this case, also. The random migration (Hank's solution in the attractant compartment) of the patient's cells was not clearly different from that of control cells. The patient's serum behaved like normal serum. The analysis made so far does not allow us to make conclusions about the true nature of the defect, which might be in the stimulation of migration by chemoattractants (chemokinesis), or in detecting the chemotactic gradient (chemotaxis).

Monosomy-7 is one of a number of non-random chromosome abnormalities occurring in disorders of the human bone marrow⁹. We have diagnosed five examples of this abnormality (Table 2). The occurrence of a cellular defect of chemotaxis, which itself is a very rare disorder, in at least four of the five can hardly be coincidental. The question arises whether the defect is due to the chromosome abnormality itself or whether it is caused by the disease. All the patients had overt leukaemia or preleukaemia. We have investigated about 30 other patients with similar diseases, many of whom had various chromosomal abnormalities¹⁰, as well as about 40 patients with infection problems, and found a cellular defect of chemotaxis in only one additional case. This was a patient with preleukaemia and trisomy-8, but in another patient with preleukaemia and trisomy-8 chemotaxis was normal¹⁰. Thus the incidence of a chemotactic defect in monosomy-7 seems to be very high compared with other patients with similar diseases.

The present results suggest that monosomy-7 is intimately

associated with defective response to chemoattractants. Although the nature of the association and the causal relationships are obscure, it seems reasonable to assume that chromosome-7 carries a gene or genes important for the chemotactic or chemokinetic response of neutrophils. This phenomenon may be similar to the loss of the Colton blood groups in monosomy-7 (ref. 11).

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Age-dependent surface marker on haemopoietic stem cells

HAEMOPOIETIC stem cells, intravenously injected into lethally irradiated mice, form colonies in their spleens (colony-forming units in spleen, CFUS)¹. This is a clonal assay²; CFUS form the precursors not only of red cells, granulocytes and platelets³ but also themselves (self-renewal)⁴. Rosendaal *et al.*⁵ reported that five spaced doses of hydroxyurea yielded femoral CFUS with a self-renewal

Table 1 Cellular products of the graft or circulating CFUS from anaemic mice

	Cell dose of graft in ml of donor blood	Colonies per spleen ±s.e.m.	DNA (μg) per spleen ±s.e.m.	DNA (μg) per colony ±s.e.m.	% ⁵⁹ Fe uptake in recipients' blood ±s.e.m. (ERA)	% ⁵⁹ Fe uptake per colony ±s.e.m.	CFUC per spleen ±s.e.m. × 10 ⁻³	CFUC per colony ±s.e.m. × 10 ⁻³	Secondary CFUS per original colony
Irradiation control	Nil	0.3±0.2	309.9±15.6	—	0.5±0.2	—	0.6±0.2	—	—
RAMB	0.4	29.3±2.4	629.9±52.0	11.0±2.1	4.6±0.6	0.14±0.02	36.3±8.5	1.2±0.3	4.6±1.5
NRS	0.2	38.0±2.9	910.0±36.2	15.9±1.6	10.5±1.0	0.27±0.03	97.3±9.7	2.6±0.3	20.1±2.0

Normal, 3-month-old female BALB/c mice were the donors of the circulating CFUS used in these experiments. To increase the number of circulating CFUS mice were rendered anaemic by three consecutive daily subcutaneous injections of phenylhydrazine (Ajax Chemicals, Sydney, 1 mg per g body weight in neutral normal saline) and bled by cardiac puncture on the fifth day⁷. CFUS in blood were partly separated from red cells by dilution (1:1 normal saline) and centrifugation (800g, 20 min, RT) over a layer of Isopaque-Ficoll (Nyegaard, Oslo and Pharmacia, Uppsala, specific gravity 1.073). The layer of mononuclear cells at the plasma-Isopaque junction was collected. There was no detectable loss of CFUS with this technique. CFUS were exposed to NRS or adsorbed (BALB/c liver, red cells and thymus) RAMB⁸, then complement (fresh frozen guinea pig serum) and finally washed before intravenous injection into recipients. Twice as many RAMB-treated cells than NRS-treated cells were injected so that the number of spleen colonies in recipients was about equal. Recipients (male BALB/c mice) were irradiated before grafting (20 per group) (700 R, 230 kVp, HVL 1 mm Cu, 146 R min⁻¹, TSD 50 cm, Maximar) and received intravenous ⁵⁹Fe as the citrate 8 d later (0.5 μCi per mouse, ferric citrate, specific activity 100 μCi per 9.7 μg Fe). Half of each group was killed 10 d after grafting, cardiac blood was taken for estimation of the erythrocyte repopulating ability (ERA)¹¹ and spleens were taken for DNA estimation and colony counts¹². 12 d after grafting the remaining mice were bled, killed and their spleens were collected for assay of CFUC and CFUS. CFUC were grown in α medium (Flow)-0.3% agar (Difco, Bacto Agar) for 14 d⁹. A single cell suspension of each spleen (passed through a sterile stainless steel sieve and gauze) was seeded—1/30 of an irradiation control spleen and 1/58 of a RAMB or NRS spleen. Dishes containing 5,000 normal nucleated myeloid cells were stimulated with the same colony-stimulating factor in the same culture and formed 100 colonies per dish. Secondary CFUS per original colony were assayed in pooled groups (irradiation control, RAMB and NRS, 10 mice per group).

capacity three times greater than that of untreated mice. A generation-age hypothesis was proposed as an explanation: the way the population of CFUS in the animal is organised to form blood is based on the generation-age of each CFUS after its origin in the animal. Younger stem cells have undergone fewer generation-ages so their capacity for self-renewal is greater. As stem cells age, the chances of their progeny being CFUS diminishes and the likelihood of their being cells committed to form red cells, granulocytes or platelets increases. It should be possible to demonstrate differences between stem cells with a reduced capacity to form themselves and those whose capacity is greater. I report here experiments with CFUS from the blood of anaemic mice treated with phenylhydrazine (PHZB CFUS)⁸, whose capacity for self-renewal is known to be reduced⁷.

PHZB CFUS were exposed to rabbit-anti-mouse-brain serum (RAMB, anti-CFUS serum)⁸, or control normal rabbit serum (NRS) and then complement before grafting. The average NRS-treated PHZB CFUS formed four times more CFUS and twice as many colony forming units in culture (CFUC, committed precursors of granulocytes, progeny of CFUS)⁹ and red cells than the average PHZB CFUS which had survived exposure to RAMB (Table 1). If allowance is made for the fact that 60% of the original PHZB CFUS had the RAMB determinant the differences were greater, threefold (CFUC and RBC) and sixfold (CFUS).

It is interesting that this same batch of RAMB killed 75% of normal bone marrow CFUS but only 50–60% (in different experiments) of PHZB CFUS. Furthermore, this RAMB had no effect on CFUC, progeny of CFUS, exposed to it before plating.

These results indicate that there are differences in the expression of the RAMB determinant between circulating stem cells with different capacities for self-renewal.

If the generation-age of stem cells were one of the ways in which blood formation were organised, it is appropriate to consider how that might be ordered in the animal. A distinction would have to be made between older and younger stem cells—an age-dependent surface marker on them might be one such means. Thus, as the stem cell aged, the expression of a receptor on it could diminish. Till has pointed out¹⁰ that RAMB, which is expressed on some stem cells but not on CFUC, is a candidate surface marker. These results are compatible with his proposal. Experiments are in progress to elucidate the function of the RAMB determinant in the animal.

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Serum and hormonal regulation of the “resting-proliferative” transition in a variant of 3T3 mouse cells

3T3 CELLS are embryonic mouse fibroblasts established in culture¹. Serum depletion or “step-down” causes them to enter a “resting” state, which they leave on restoration of serum or “step-up”, entering the “proliferative” state^{2–4}. This transition between resting (also called G₀, A or R) and proliferating states seems to be the critical step in cell growth control^{5–7}. Its study has been aided recently by several observations on extracellular growth regulators for 3T3 cells, for example the discovery of a new pituitary growth factor^{4,8}, growth stimulation by glucocorticoids^{4,9} and interactions between pituitary factor and classical hormones^{10–12}. Further studies would be greatly helped if variants or mutants of 3T3 with different growth responses towards hormones could be isolated. Here we report our finding of the variant ST1 which is inhibited by glucocorticoids, unlike 3T3 cells whose growth is stimulated by glucocorticoids^{4,9} (growth of fibroblasts is normally inhibited by glucocorticoids¹³). In this paper we discuss the value of this new cell type for studies of the resting-proliferative transition; elsewhere we shall report how the

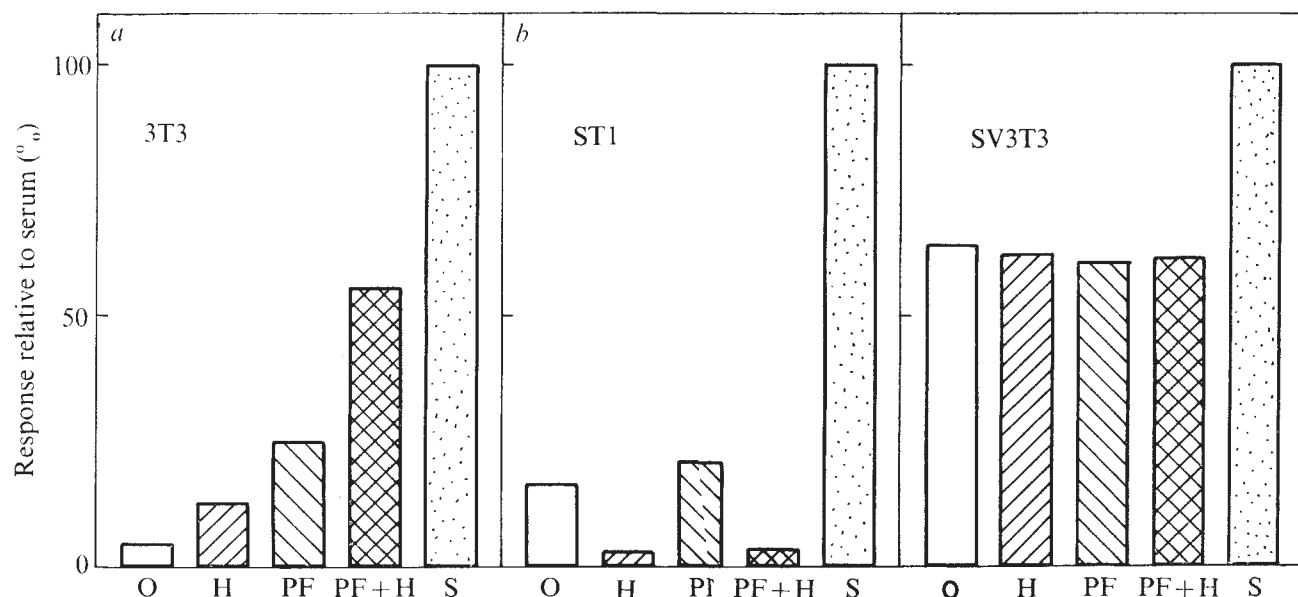
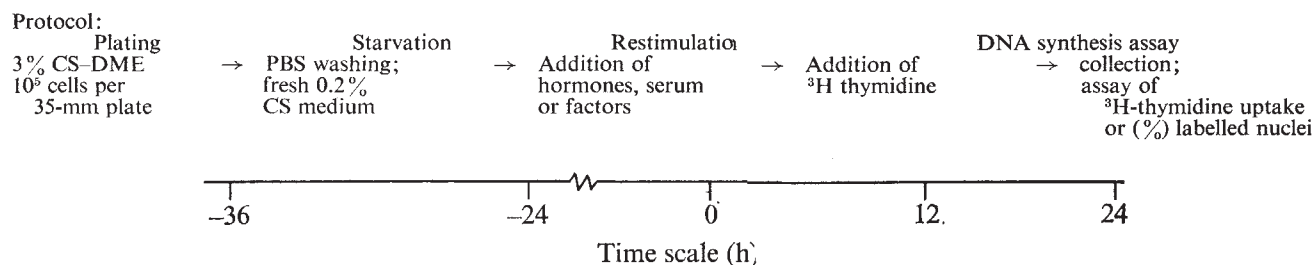


Fig. 1 Effect of hydrocortisone (H), pituitary factor (PF) and calf serum (S) on ^3H -thymidine uptake ($0.25 \mu\text{C ml}^{-1}$, 10^{-7} M) into DNA for three different clones of Swiss 3T3 cells (American Type Culture Collection).



▲ Abscissa shows additions to 0.2% calf serum at $t=0$. Two plates per point. Deviation was usually 5% and never greater than 10%. ^3H -thymidine uptake into DNA was as described in ref. 4. 100% corresponds to 3.3×10^4 c.p.m. per plate for clone 1; 4.9×10^4 c.p.m. per plate for ST1 and 5.1×10^4 c.p.m. per plate for SV3T3. PF is a growth factor preparation from frozen bovine pituitary glands containing seven bands in polyacrylamide gel electrophoresis (ref. 19).

adhesion of this variant to the substrate is greatly affected by both the removal of serum and the addition of cyclic AMP.

We sought new cell types by cloning mutagenised and non-mutagenised 3T3 populations; routinely confluent monolayers cultures were inspected for foci of piled-up variants. Other selection procedures have also been developed which will be reported elsewhere. In one particular case, repeated plating in low serum of non-mutagenised culture containing foci yielded a population enriched with variant cells which was then cloned and re-cloned. Since the variant clones obtained were all identical, one was chosen and called ST1 and the others were discarded.

Figure 1 represents the typical response of clones isolated from the parental 3T3 line, the ST1 variant and SV40-transformed 3T3 (SV3T3).

As can be seen from Table 1 and Fig. 1b, ST1 cells have: (1) diminished density inhibition of growth—they pile up and grow in methocel; (2) serum dependence; (3) half as many chromosomes as the parental line. Also their synthesis of DNA is not dependent on pituitary factor but is inhibited by hydrocortisone. In addition, insulin (at physiological or high doses) and a somatomedin-enriched serum factor have no effect on DNA synthesis but factor(s) contaminating commercial crystalline bovine serum albumin cause stimulation (results not shown).

The hydrocortisone inhibition of growth has the following very interesting features: (1) it is specific for glucocorticoids (sex steroids have no effect); (2) glucocorticoids are effective at physiological doses; maximal effect is obtained with 20 ng ml^{-1} dexamethasone or 50 ng ml^{-1} hydrocortisone; (3) the glucocorticoid growth inhibition depends on serum

Table 1 Properties of 3T3, ST1 and SV 3T3 cells

Cell line	Saturation density (cells cm^{-2})		Doubling time (h)		Growth in methocel	No. of chromosomes per cell
	10% CS	1% CS	10% CS	1% CS		
3T3	0.4×10^5	0.9×10^4	18	0	—*	80 ± 2.5
ST1	4.1×10^5	2.5×10^4	14.5	3	+†	40 ± 1
SV 3T3	4.5×10^5	2.3×10^4	13	8	+	75.6 ± 4.8

Saturation density and doubling time were determined using the same batch of calf serum. Cells were plated in methocel according to ref. 20. Karyotype analysis was done according to ref. 21. At least 10 metaphase plates were counted per cell line; figures are the mean $\pm$ s.e.m.

*No visible colonies after one month of culture; eventually presence of microcolonies of up to eight cells; plating density from 10^6 to 10^8 cells per plate; efficiency of plating: zero.

†Visible (2–4 mm) colonies counted 21 d after plating; efficiency of plating in different experiments: 1 to 3%.

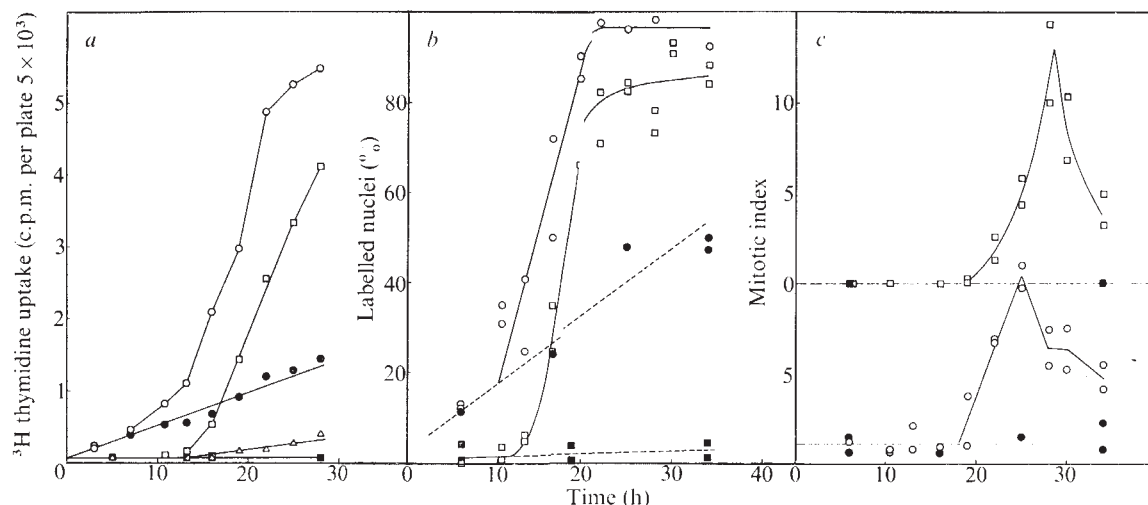


Fig. 2 Kinetics of restimulation with serum for ST1 cells treated with glucocorticoid and untreated. Cells were plated in 3% calf serum; 24 h later the medium was changed to 0.2% calf serum plus or minus hydrocortisone (300 ng ml⁻¹). After 24 h incubation in low serum, calf serum (5%) was added to restimulate cells. Plates treated with hydrocortisone were washed with PBS and received fresh 0.2% calf serum medium before serum addition. ³H-thymidine (0.25 μCi ml⁻¹, 5 × 10⁻⁷ M) was added to all plates at the same time as serum. Plates were harvested at different time intervals after serum addition and assayed for: *a*, ³H-thymidine uptake into DNA; *b*, percentage of labelled nuclei; *c*, mitotic index. ○, Plus serum, not treated with hydrocortisone; ●, no serum added, not treated; □, treated with hydrocortisone, steroid washed away, plus serum; ■, treated with hydrocortisone, steroid washed away, no serum added; △, treated with hydrocortisone, no serum added, steroid not washed away. Percentage of labelled nuclei determined by autoradiography using Kodak AR10 stripping film. Mitotic index determined in fixed and stained plates. Two plates per point for *a* (deviation never greater than 10%) one plate per point for *b* and *c* (at least 500 cells counted per plate in *b* and at least 1,000 cells per plate in *c*).

concentration: at 0.2% serum inhibition is 100% whereas at 5% serum it is 40%; (4) inhibition is completely reversible and glucocorticoid has no effect on cell viability: plating efficiency is the same (50–55%) in the presence or absence of hydrocortisone; (5) the inhibition, measured by 1-h ³H-thymidine pulses, increases progressively with time after glucocorticoid addition, attaining its maximal level after 15–18 h of glucocorticoid addition; (6) glucocorticoids had no detectable effect on ongoing DNA synthesis or the ability of cells to undergo mitosis (data not shown); (7) 10% of the cells from an exponentially growing culture are found in a “resting” state 15–20 h after serum step-down in the presence of hydrocortisone; these “resting” cells are fully responsive on serum restimulation (Fig. 2).

The results of kinetic experiments, shown in Fig. 2, in which ³H-thymidine labelling and mitotic index were followed, indicate that: (1) serum step-down causes accumulation of cells at the beginning of G₁ or at G₀, though in the absence of hydrocortisone residual DNA synthesis and growth remain; (2) from the kinetics of the stimulated DNA synthesis one can make estimates of the length of G₁ (Table 2): in the presence of hydrocortisone G₁ was longer by 4.0 h (³H-thymidine uptake) or 3.8 h (labelled nuclei) than in its absence; (3) the increment in mitotic index was delayed by 3.8 h (Table 2) in cells treated with hydrocortisone, indicating that hormone-related and untreated cells traverse S and G₂ at the same rate.

In conclusion, it seems clear to us that glucocorticoids at physiological doses inhibit growth of ST1 cells without compromising their viability or interfering with biochemical processes of S, G₂ or mitotic phase of the life cycle. This inhibitory effect seems to be directly connected with the mechanism which regulates the transition between “resting” and “proliferative” states of the cell.

Resting “normal” 3T3 cells when restimulated by serum step-up or PF addition enter the S phase, after identical G₁. Addition of hydrocortisone to PF further increases the stimulation, accelerating the entry of cells into S, but without interfering with G₁ length (M.C.S.A. and H.A.A., unpublished). This accelerating effect is due to an increase in the probability of cells exiting in the resting state. On the other hand the glucocorticoid seems to cause a resetting of the growth control mechanisms of ST1 cells, leading to a strict “resting” state with serum step-down and a larger G₁ upon restimulation by serum step-up. We feel that our approach to the growth regulation of 3T3 cells and its variants might yield new information about the mechanisms of cell growth control.

It has long been recognised that glucocorticoids inhibit the growth of fibroblasts *in vivo* and *in vitro*¹³, but the mechanisms of this effect are still unknown. In culture this glucocorticoid-inhibiting effect has been studied using L cells, a line of tumorigenic mouse fibroblasts^{14–17} and has also been observed with normal chick embryo cells¹⁸. It

Table 2 Estimates of G₁ and T (G₁+S+G₂) on restimulation of ST1 cells with serum

Kinetics of	G ₁ ⁺ ^H	G ₁ ^{-H}	(G ₁ ⁺ ^H —G ₁ ^{-H})	T ⁺ ^H	T ^{-H}	(T ⁺ ^H —T ^{-H})
³ H-thymidine uptake into DNA	1) 14.5	1) 10.4	1) 4.1	—	—	—
	2) 14.1	2) 10.6	2) 3.9	—	—	—
Nucleus labelling	2) 13.7	2) 10.1	2) 3.8	—	—	—
Mitotic index	—	—	—	2) 21.8	2) 18.0	2) 3.8

G₁⁺^H and G₁^{-H} are the length of G₁ (in hours) for cells treated with hydrocortisone and untreated, respectively. T⁺^H and T^{-H} are the period of time equivalent to G₁+S+G₂ for treated and untreated cells, respectively. G₁ was estimated by taking the best-fitting straight lines from kinetic curves like the ones shown in Fig. 2*a* and *b* in the interval 0 to 22 h for non-stimulated and 11 and 22 h for stimulated cultures. The abscissa corresponding to the intercept of these two lines was taken as G₁ (or the length of time a cell takes to leave the resting state and initiate DNA synthesis or enter the S phase, see refs 5, 6 and 7). Estimates of T were obtained in a similar manner using mitotic index curves like the ones shown in Fig. 2*c*. 1 and 2 correspond to estimates obtained from two completely independent experiments.

would be interesting to find out whether the pattern of inhibition we describe for ST1 cells is a common response for fibroblasts. Such a suggestion is not unreasonable considering the results reported for chick cells¹⁸ and L cells¹⁷.

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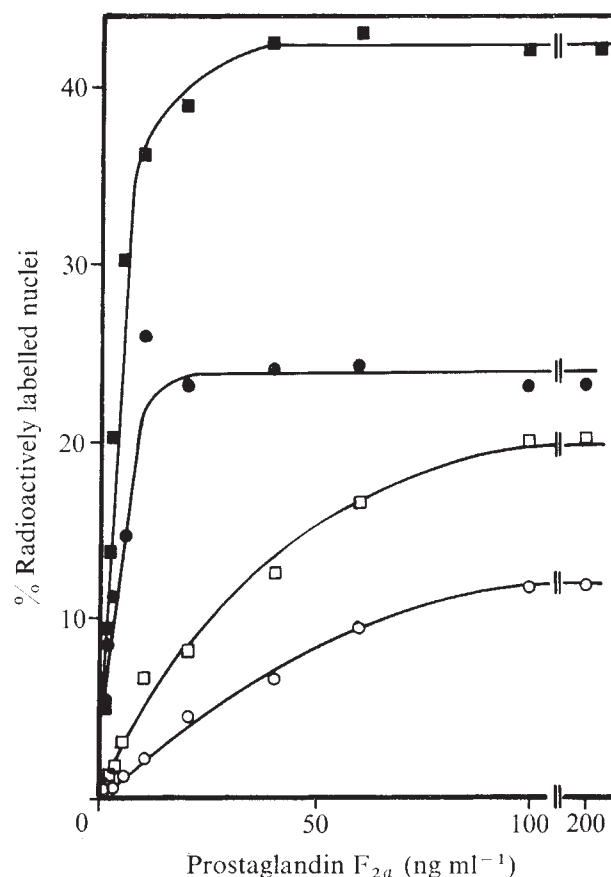


Fig. 1 Effect of different concentrations of $\text{PGF}_{2\alpha}$ on the percentage of cells synthesising DNA in 30 h. Increasing concentrations of $\text{PGF}_{2\alpha}$ were added to quiescent 3T3 cells in Dulbecco's modified Eagles medium (DEM) without ($\circ$ — $\circ$) and with ($\bullet$ — $\bullet$) 50 ng ml^{-1} insulin or to quiescent cells in DEM supplement with low molecular weight nutrients (DMS) without ($\square$ — $\square$) and with ($\blacksquare$ — $\blacksquare$) insulin. Swiss mouse 3T3-4 fibroblasts⁹ were maintained in DEM containing 100 units ml^{-1} penicillin, 100 ng ml^{-1} streptomycin and 10% foetal calf serum. Subconfluent cultures were grown in 90 mm Nunc Petri dishes at 37 °C under a pressure of 10% CO_2 and were routinely checked for any contamination by mycoplasma⁷. Cells were plated at 10^5 per 33 mm dish in 2 ml of DEM and 6% foetal calf serum, or in DEM and 6% serum supplemented with 0.03 μM biotin, 0.05 μM vitamin B_{12} , 140 μM aspartic acid, 0.3 mM glutamic acid, 50 μM glutathione, 60 μM hypoxanthine. All additional nutrients were required to obtain the maximum stimulation of DNA synthesis by $\text{PGF}_{2\alpha}$ and insulin in 3T3 cells. All additions were made at the same time and then 3 $\mu\text{Ci ml}^{-1}$ methyl- ^3H -thymidine was added at 1 μM and the cells were left for 30 h. Cultures were then processed for autoradiography as previously described^{6,7}, and the percentage of cells with radioactively labelled nuclei was recorded. In parallel experiments cells were radioactively labelled with 3 $\mu\text{Ci ml}^{-1}$ ^3H -thymidine at 3 μM for the same time and the radioactivity incorporated into DNA^{6,7} was determined for no additions (1,500 c.p.m.); 200 ng ml^{-1} $\text{PGF}_{2\alpha}$ (26,500 c.p.m.); $\text{PGF}_{2\alpha}$ and 50 ng ml^{-1} insulin (87,300 c.p.m.) in DEM; or no additions (1,800 c.p.m.); $\text{PGF}_{2\alpha}$ (70,000 c.p.m.); $\text{PGF}_{2\alpha}$ and insulin (144,000 c.p.m.) in DMS.

Interaction of two hormones and their effect on observed rate of initiation of DNA synthesis in 3T3 cells

THE rate of replication of mammalian cells changes in response to alterations in the external environment usually by expansion or contraction of the average time of the G_1 phase of the cell cycle; the remaining phases are relatively constant (S, G_2 , M)¹⁻³. Thus, when varying concentrations of serum⁴ or the growth-promoting⁵⁻⁷ factors are added to relatively quiescent fibroblastic cells variations in rates of cell division are mainly due to differences in rates of initiation of DNA synthesis^{4,6}. Recently Brooks⁸ has shown that addition of serum and adenosine to quiescent BHK fibroblasts stimulated the cells to enter S phase with first-order kinetics, although without adenosine the kinetics were more complicated. We now report that the stimulation of DNA synthesis induced by prostaglandin $\text{F}_{2\alpha}$ ($\text{PGF}_{2\alpha}$) can be described as an increase in a first-order rate constant for exit from the G_1 phase preceded by a lag phase of relatively fixed duration. Insulin and certain nutrients which increase the effect of $\text{PGF}_{2\alpha}$ upon DNA synthesis do so by changing the same rate constant.

Swiss mouse 3T3 fibroblastic cells⁹ were allowed to grow and become quiescent either in Dulbecco's modified Eagle's medium (DEM) and 6% serum or in the same supplemented with additional nutrients (DMS) (Fig. 1). Addition of increasing concentrations of $\text{PGF}_{2\alpha}$ (0–200 ng ml^{-1}) to the cultures caused an increasing percentage of cells to synthesise DNA in 30 h (labelling index) up to a maximum, at 200 ng ml^{-1} of 12% or 23% in DEM or DMS respectively. Addition of a physiological concentration (50 ng ml^{-1}) of insulin alone induced only 4% or 5% of the 3T3 cells in DEM or DMS to synthesise DNA. However, addition of insulin markedly increased the response to all concentrations of $\text{PGF}_{2\alpha}$ in either medium up to a maximum labelling index of 24% and 43% in DEM or DMS respectively (synergistic effect) (Fig. 1).

Two concentrations of $\text{PGF}_{2\alpha}$ (30 and 300 ng ml^{-1}) with or

without insulin were added to quiescent cultures and the cumulative labelling index was subsequently followed with time (Fig. 2). The same results were plotted as the logarithm of the percentage of unlabelled cells against time⁸. Since most of the quiescent cells (95%) were in G_1 ¹⁰ the gradient of the curves in Fig. 3 gave the first order rate constant at which G_1 cells were entering the S phase at a given time. Few cells became radioactively labelled in the first 15–16 h (lag phase). The duration of the lag phase was independent of the concentration of $\text{PGF}_{2\alpha}$ with or without insulin in DEM or DMS, and in accordance with previous results for varying serum⁴ or FGF ⁶ concentrations. At the end of the lag phase however, the rate at which cells entered S increased abruptly and within 2 h the rate constant reached a steady value (Fig. 3). Straight lines

fitted the data well indicating an underlying first-order reaction or event defined by an apparent rate constant k . The goodness of fit of regression lines to the data of Fig. 3a and b was estimated by calculating coefficients of determination r^2 . These ranged from 0.93 to 0.98 where $r^2 = 1$ implies a perfect fit. PGF_{2α} at a concentration of 30 ng ml⁻¹ in DMS gave $k = 6.0 \pm 0.5 \times 10^{-3} \text{ h}^{-1}$ while at 300 ng ml⁻¹ $k = 1.5 \pm 0.1 \times 10^{-2} \text{ h}^{-1}$. Insulin with either 30 or 300 ng ml⁻¹ of PGF_{2α} dramatically increased k to a value of $3.5 \pm 0.2 \times 10^{-2} \text{ h}^{-1}$. When 300 ng ml⁻¹ PGF_{2α} and insulin were added to cells which had become quiescent in DEM or DMS the value of k was $2.2 \pm 0.1 \times 10^{-2} \text{ h}^{-1}$ in DEM and $5.1 \pm 0.3 \times 10^{-2} \text{ h}^{-1}$ in DMS. The corresponding value for 10% serum in DMS was approximately four times as high ($k = 0.24 \pm 0.05 \text{ h}^{-1}$). Variations in absolute k values for additions of these hormones were sometimes observed for different batches of cells and serum, but the kinetics were always first order. When quiescent cells in DEM were radioactively labelled for up to 5 d (not shown) a slight but constant gradient was observed ($k = 6.4 \pm 0.5 \times 10^{-4} \text{ h}^{-1}$), presumably due to a slow rate of cell division (R. Shields and J. A. Smith, personal communication). Thus, there was probably a similar low rate of initiation of DNA synthesis during the lag phase (Fig. 3). Time-lapse cinematography of cultures indicated that cell death and detachment were occurring and this probably balanced the slow rate of cell division (R. Shields, unpublished results). When PGF_{2α} and insulin were not added together, but insulin was added 9 h later, the kinetics of entry into S were still first order and the rate constants were similar (Fig. 3c). However, when insulin was added at the end of the lag phase,

Fig. 2 Changes in the labelling index with time after addition of PGF_{2α} and insulin. The following additions were made: PGF_{2α}, 30 ng ml⁻¹ (○—○); PGF_{2α}, 30 ng ml⁻¹ + insulin, 50 ng ml⁻¹ (●—●); PGF_{2α}, 300 ng ml⁻¹ (□—□); and PGF_{2α}, 300 ng ml⁻¹ + insulin, 50 ng ml⁻¹ (■—■). The cells were grown in DMS and 6% foetal calf serum and allowed to become quiescent (Fig. 1). After additions the cultures were exposed to ³H-thymidine from 0 until the indicated times. Pairs of cultures were then processed for autoradiography and the total fraction of cells synthesising DNA in that time period (labelling index) was recorded.

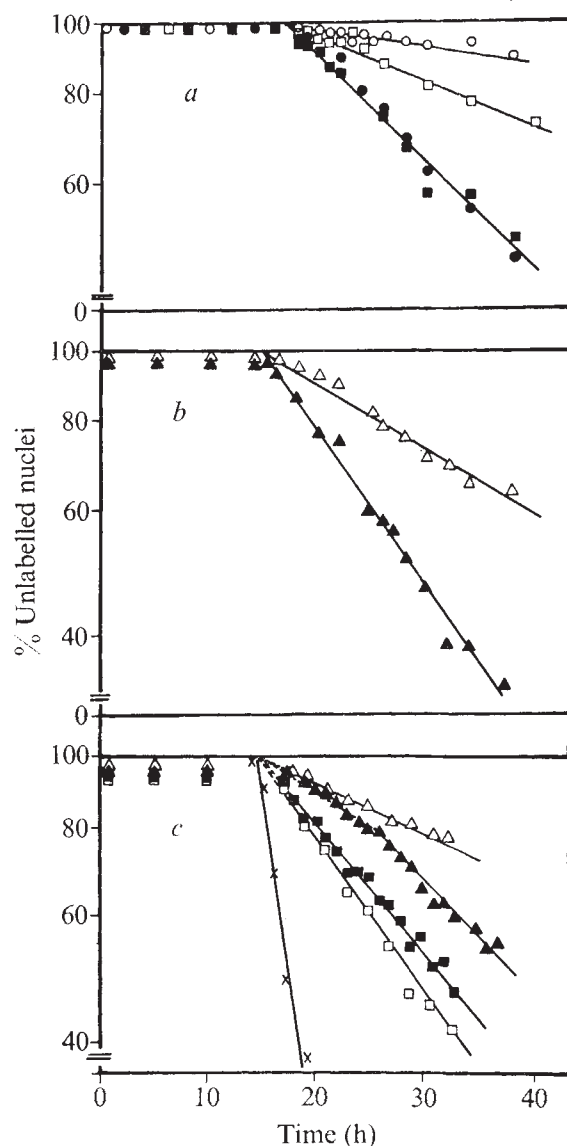
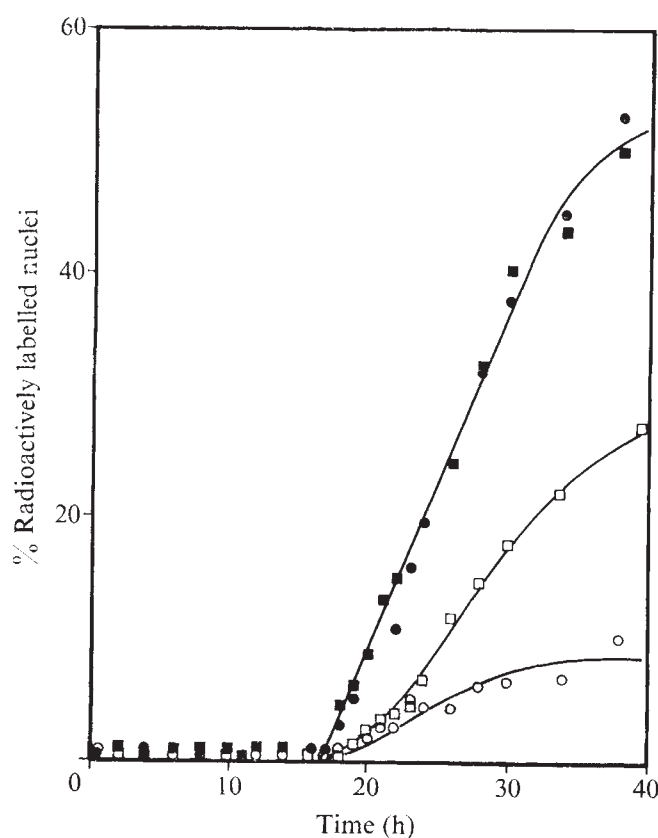


Fig. 3 a, Fraction of cells remaining in G₁ after synchronous addition of PGF_{2α} and insulin. The results in Fig. 2 were plotted as the logarithm (base 10) of the percentage of cells remaining in G₁ (percentage of non-labelled cells) $\log_{10} y$ against time (t) in hours (see below). The symbols were as follows: PGF_{2α}, 30 ng ml⁻¹ (○—○); PGF_{2α}, 30 ng ml⁻¹ + insulin, 50 ng ml⁻¹ (●—●); PGF_{2α}, 300 ng ml⁻¹ (□—□); and PGF_{2α}, 300 ng ml⁻¹ + insulin, 50 ng ml⁻¹ (■—■). Regression lines given by $\log_{10} y = a - bt$ were subsequently calculated for times greater than 15 h. The constants (a , b), with standard errors, were for PGF_{2α}, 30 ng ml⁻¹; PGF_{2α}, 30 ng ml⁻¹ + insulin; PGF_{2α}, 300 ng ml⁻¹; PGF_{2α}, 300 ng ml⁻¹ + insulin: $(2.029 \pm 0.004, 0.0026 \pm 0.0002)$; $(2.268 \pm 0.017, 0.0154 \pm 0.0007)$; $(2.121 \pm 0.013, 0.0066 \pm 0.0005)$; $(2.248 \pm 0.017, 0.0148 \pm 0.0007)$. Testing the goodness of fit yielded values of coefficients of determination (r^2) of 0.93, 0.98, 0.96 and 0.98 respectively. b, Fraction of cells remaining in G₁ in DEM and DMS after addition of PGF_{2α} and insulin. PGF_{2α}, 300 ng ml⁻¹ and insulin, 50 ng ml⁻¹ were added to quiescent cultures grown in DEM (△—△) or DMS (▲—▲) and 6% serum. Cultures were processed as described in Fig. 2 and plotted as above. The constants (a , b), with standard errors, for the regression lines $\log_{10} y = a - bt$, were for DEM and DMS: $(2.150 \pm 0.015, 0.0096 \pm 0.0006)$ and $(2.332 \pm 0.037, 0.0221 \pm 0.0014)$ while $r^2 = 0.96$ and 0.97 respectively. c, Fraction of cells remaining in G₁ after non-synchronous additions of PGF_{2α} and insulin. PGF_{2α}, 300 ng ml⁻¹ was added to quiescent cultures grown in DMS and 6% serum. Insulin (50 ng ml⁻¹) was added at various times afterwards, as follows: no insulin (—△—△—); insulin at 17 h (—▲—▲—); insulin at 9 h (—■—■—); insulin at start with PGF_{2α}, (—□—□—). Controls with fresh 10% serum added at the start, (—×—×—). Cultures were processed and the results plotted as above. The values for the first-order rate constants k for addition of PGF_{2α} with no insulin; with insulin after 17 h; with insulin after 9 h; and with insulin at the start were: 1.6 ± 0.3 ; 3.3 ± 0.7 ; 3.9 ± 0.5 ; and $4.7 \pm 0.8 \times 10^{-2} \text{ h}^{-1}$ respectively. For 10% serum from the start $k = 0.24 \pm 0.05 \text{ h}^{-1}$.

Table 1 Stimulation of rate of 2-deoxyglucose uptake, rate of cellular protein synthesis and increase in rate constant (k)

Additions	2-deoxyglucose* (%)		Protein synthesis† (%) 5 h		Rate constant (k) for entry into S‡ (%)
	1 h	5 h	Methionine	All amino acids	
None	100	100	100 ± 4	100 ± 9	100
Insulin	312	372	124 ± 8	107 ± 6	310
PGF _{2α}	110	565	127 ± 3	120 ± 4	740
PGF _{2α} + insulin	340	1,890	206 ± 11	198 ± 4	1,740
Serum	350	3,758	228 ± 8	210 ± 4	—

3T3 cultures were grown and allowed to become quiescent in DEM as in Fig. 1. Then 300 ng ml⁻¹ of PGF_{2α}, 50 ng ml⁻¹ insulin, 10% foetal calf serum were added as indicated. After 45 min or 4 h 45 min, 2-deoxyglucose uptake was measured¹⁹. The cell monolayers were washed three times with prewarmed serum-free medium without glucose and then incubated for 15 min in the medium with hormones or serum. The cells were then exposed to 3 μCi ml⁻¹ of 2-deoxyglucose at 50 μM for 15 min. Uptake into the fraction soluble in 5% trichloroacetic acid was linear for this time¹⁹.

*Results expressed relative to the incorporation for no additions (320 c.p.m. per min per mg protein). Protein synthetic rates were determined by exposing the cultures to either 10 μCi ml⁻¹ ³⁵S-methionine or 0.5 μCi ml⁻¹ ¹⁴C total amino acids in the normal medium from 2 h until 5 h after the additions and then removing samples every 30 min up to 6 h after adding the isotope. Cell monolayers were washed twice with isotonic saline, dissolved in 2.0 ml of 0.5 M NaOH and divided into two equal fractions. Total protein content was determined in one fraction²⁰, while the second fraction was incubated 1 h at 37 °C to hydrolyse the aminoacyl tRNAs. This was then precipitated with trichloroacetic acid and analysed for radioactivity²¹. The incorporation of radioactivity into the TCA-soluble pool was constant at 3 and 6 h and independent of hormonal additions, and the incorporation of radioactivity into protein per mg cell protein was linear with time for the 6-h interval²¹.

†Results expressed as a percentage of the value for no additions (³⁵S-methionine, 100% = 53,500 c.p.m. per h per mg protein; ¹⁴C total amino acids 100% = 7,300 c.p.m. per h per mg protein). Results ± standard errors are the means of the rates of incorporation measured over 6 h in duplicate experiments.

‡The rate constant (k) for cellular entry into S was calculated from the radioactive labelling index at 30 h assuming k to be constant and is expressed as a percentage of the value for no additions (100% = 0.10% of cells entering S h⁻¹). No value of k was calculated for serum addition since the rate of entry into S does not follow first-order kinetics under these conditions⁸, but the labelling index at 30 h was 88%.

the rate constant gradually increased. After 9 or 10 h the rate constant was nearly the same as that for simultaneous additions. This suggested that when insulin was added near the completion of the PGF_{2α}-induced lag phase changes in the rate constant for entry into S could be produced without appreciable delay.

PGF_{2α} and insulin separately increased protein synthetic rates about 1.2-fold, but when added together they increased the rates by a maximum of about 2-fold after 5 h (Table 1). Synergistic effects of PGF_{2α} and insulin were observed in the protein synthesis-dependent increase in 2-deoxyglucose uptake (Table 1) which also started after 2–6 h. The latter increases were much larger (19-fold) than those in total protein synthesis and closely reflected the increased rate constants for entry into S (ref. 12) (Table 1). No synergistic effects, however, were observed in the initial protein synthesis-independent increases in 2-deoxyglucose (Table 1) rubidium¹³ or phosphate¹³ uptakes.

Our results are compatible with the two models of the cell cycle proposed by Smith and Martin¹⁴ and by Pardee¹⁵. In the former model cells enter an indeterminate state (A state) in G₁. From this they proceed to the determinate phase (B phase) of the cell cycle (S, G₂, M and remainder of G₁) at random with a constant probability (P). This predicts first-order kinetics of initiation of DNA synthesis in constant conditions. The growth stimulation of quiescent cells is then a switching of the cell population from one A state to another of higher P . In the second model the major growth regulatory point in a cell exists as a single restriction point (R point) in G₁ for critical nutrients and growth-stimulatory agents. Readdition of these components to deprived cells results in a constant lag phase prior to DNA synthesis.

A single, rate-limiting step or event is strongly suggested by the first-order kinetics of cellular entry into S phase. This step may occur close to the onset of DNA synthesis¹⁶. As shown above, PGF_{2α} and insulin interact synergistically upon the rate of the cells' entry into the S phase, and thus presumably upon that step. Interaction also occurs in some of their effects involving protein synthesis although not always at their first presumed site of action in the plasma membrane^{17,18}. It is thus possible that the interaction upon DNA synthesis may, like that upon 2-deoxyglucose uptake, depend on synergy upon synthesis of protein(s). This is not inconsistent with the ability of insulin when added later than PGF_{2α} to affect the initiation of DNA synthesis within a few hours. As the duration of the

lag phase appears independent of serum^{4,6,8,11}, or hormonal concentrations⁶ the events determining this duration may be partly different from those by which hormonal concentrations determine the rate of initiation of DNA synthesis.

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Cyclic AMP regulation of mitogen-induced interferon production and mitogen suppression of immune response

In a recent review, adenosine 3',5'-cyclic monophosphate (cyclic AMP) was proposed to have an inhibitory effect on the immunological and inflammatory functions of leukocytes¹. Guanosine 3',5'-cyclic monophosphate (cyclic GMP) has been shown to reverse the inhibitory effect of cyclic AMP in the *in vitro* immune response, and it has been proposed that the ratio of cyclic AMP to cyclic GMP determines whether an immune response will be enhanced or suppressed². Cyclic AMP has recently been shown to inhibit the production of several soluble lymphocyte mediators (lymphokines) under antigen or mitogen stimulation³⁻⁵. T lymphocyte mitogens are potent inhibitors of the *in vitro* immune response as a result of stimulation of suppressor cell activity⁶⁻⁸. They are also potent stimulators of the synthesis of a unique type of interferon by T lymphocytes⁹⁻¹¹. Evidence suggesting that this interferon may be the mediator of some forms of suppressor T cell activity has been presented¹¹. I examine in this report the ability of exogenous cyclic AMP, and agents that increase the endogenous level of cyclic AMP, to block both the production of mitogen-type interferon and the development of the suppressor state in an *in vitro* murine antibody system. The data show that cyclic AMP blocks both aforementioned T cell functions, and are consistent with the hypothesis that interferon may be the mediator of some forms of suppressor cell activity¹¹.

Figure 1 presents data showing dibutyryl cyclic AMP inhibition of the production of interferon in C57B1/6 mouse spleen cell cultures that were stimulated by the two T cell mitogens concanavalin A (con A)¹² and staphylococcal enterotoxin A (SEA)⁸. Dibutyryl cyclic AMP, 2×10^{-5} M, inhibited con A stimulation of interferon production by 96%, while 1×10^{-4} M inhibited SEA stimulation of interferon production by 85%. When the same concentrations of dibutyryl cyclic AMP were added to the supernatant fluids of mouse spleen cell cultures after complete interferon production, no inhibition of antiviral activity of the interferon was observed, so it is concluded that dibutyryl cyclic AMP blocked the production of interferon, and not the established activity of produced interferon.

Table 1 presents data on dibutyryl cyclic AMP blockade of mitogen-induced suppression of the *in vitro* plaque-forming cell response to sheep red blood cells (SRBC). SEA

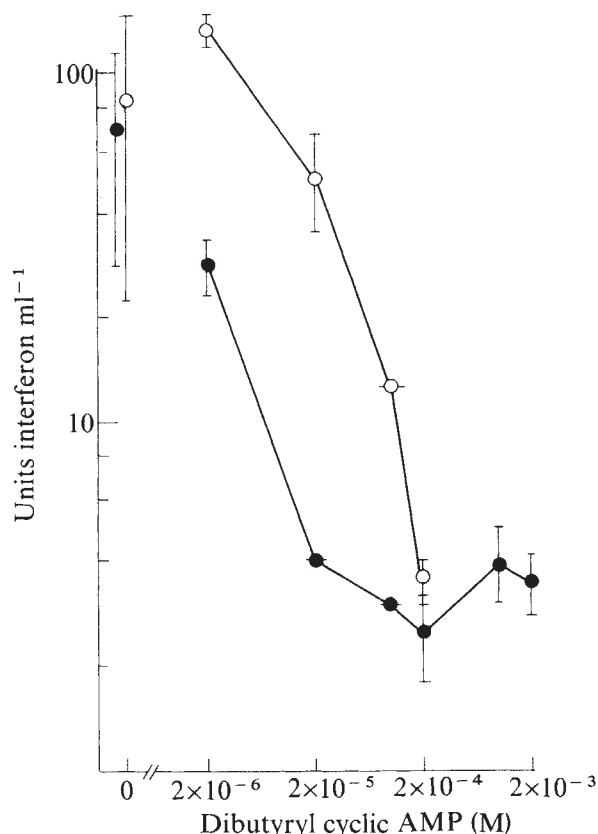


Fig. 1 Dibutyryl cyclic AMP inhibition of the production of interferon in concanavalin A (con A, ○) and staphylococcal enterotoxin A (SEA, ●) stimulated C57B1/6 female (8 week old) mouse spleen cell cultures. Cultures consisted of 1.5×10^7 spleen cells per ml and $2 \mu\text{g}$ and $1 \mu\text{g}$ con A and SEA, respectively. The con A and SEA are the same mitogens used previously¹¹. Dibutyryl cyclic AMP was obtained from Calbiochem, San Diego, California. Dibutyryl cyclic AMP and mitogens were added to cultures in modified minimal essential media (MEM) (100- μl volumes) at the same time and the cells were incubated for 48 h as previously described¹¹. Supernatant fluids were obtained by centrifugation of the collected cultures at 1,000 r.p.m. in a RC-3 Sorvall centrifuge at 7 °C. Fluids were assayed on the day of collection for mitogen-induced interferon activity in mouse L cells by a slight modification of the method of inhibition of cytopathic effect of vesicular stomatitis virus (100 TCID₅₀ per challenge dose)¹³. Data are plotted as units of interferon $\text{ml}^{-1} \pm \text{s.d.}$ for duplicate samples.

Table 1 Effect of dibutyryl cyclic AMP on mitogen-induced suppression of the *in vitro* PFC response to SRBC

Mitogen	Mitogen concentration ($\mu\text{g ml}^{-1}$)	Dibutyryl cyclic AMP (1.4×10^{-4} M)	PFC per culture $\pm \text{s.d.}$	PFC per 10^6 viable cells $\pm \text{s.d.}$
SEA	0.25	—	350 $\pm$ 71	64 $\pm$ 8
SEA	0.50	—	550 $\pm$ 71	101 $\pm$ 11
SEA	0.25	+	13,150 $\pm$ 2051	5,080 $\pm$ 820
SEA	0.50	+	13,500 $\pm$ 1414	5,508 $\pm$ 1406
—	—	+	9,650 $\pm$ 2616	4,148 $\pm$ 296
—	—	—	18,350 $\pm$ 4596	5,206 $\pm$ 3626
Con A	1.0	—	25 $\pm$ 35	7 $\pm$ 9
Con A	2.0	—	100 $\pm$ 71	16 $\pm$ 11
Con A	1.0	+	1,900 $\pm$ 566	1,577 $\pm$ 505
Con A	2.0	+	1,750 $\pm$ 424	1,456 $\pm$ 307
—	—	+	9,775 $\pm$ 1308	3,146 $\pm$ 537
—	—	—	15,325 $\pm$ 5409	2,571 $\pm$ 691

Dissociated normal female (8 week old) C57B1/6 mouse spleen cells were cultured for *in vitro* anti-SRBC plaque-forming cell (PFC) response exactly as described by Mishell and Dutton¹⁴. Cultures consisted of 1 ml of 1.5×10^7 spleen cells ml^{-1} and 3×10^6 SRBC. Dibutyryl cyclic AMP was added to cultures 1 h before mitogen and SRBC addition. Dibutyryl cyclic AMP and mitogens were diluted in modified MEM, and added to cultures in 100 μl volumes. All PFC responses were determined at day 5. Cell viabilities were determined by trypan-blue dye exclusion. Direct PFC assays were carried out on microscope slides as previously described¹⁵. Results are expressed as the average of duplicate cultures $\pm \text{s.d.}$

Table 2 Effect of agents that increase endogenous cyclic AMP levels on SEA-induced suppression of the *in vitro* PFC response to SRBC

Agent ($\mu\text{g ml}^{-1}$)	SEA ($0.5 \mu\text{g ml}^{-1}$)	PFC per culture $\pm$ s.d.	PFC per 10^6 viable cells $\pm$ s.d.
—	+	1,625 $\pm$ 247	431 $\pm$ 135
Cholera toxin (0.1)	+	9,600 $\pm$ 1061	2,984 $\pm$ 596
Cholera toxin (0.1)	—	7,225 $\pm$ 1096	4,791 $\pm$ 165
—	—	13,150	4,326
—	+	500 $\pm$ 71	91 $\pm$ 42
IMX (12.5)	+	5,300 $\pm$ 424	3,973 $\pm$ 477
IMX (12.5)	—	3,500 $\pm$ 1556	1,366 $\pm$ 571
—	—	9,850 $\pm$ 283	2,294 $\pm$ 1238

Cholera toxin and IMX (3-isobutyl-1-methyl xanthine) were added to *in vitro* plaque-forming cell (PFC) cultures under the same conditions as described in Table 1. IMX was obtained from Aldrich Chemical Company, Milwaukee, Wisconsin. Results are expressed as the average of duplicate cultures $\pm$ s.d., except where only one culture was assayed.

at 0.25 and $0.50 \mu\text{g ml}^{-1}$ inhibited the plaque-forming cell response by $>90\%$ when compared to cultures receiving either SRBC alone or dibutyryl cyclic AMP along with the SRBC. Protection of the plaque-forming cell response was essentially complete when dibutyryl cyclic AMP (1.4×10^{-4} M) was added to cultures along with SEA. The protection of the anti-SRBC plaque-forming cell response was significant when expressed either as plaque-forming cells per culture or plaque-forming cells per 10^6 viable cells. Dibutyryl cyclic AMP protection of the plaque-forming cell responses from con A suppression was also observed. Con A at 1.0 and $2.0 \mu\text{g ml}^{-1}$ inhibited the plaque-forming cell response by $>90\%$ when compared to the controls. Blockade of suppression was more dramatic when the plaque-forming cell response was expressed in terms of viable cells recovered, since the presence of con A and dibutyryl cyclic AMP in the same culture resulted in relatively fewer viable cells recovered as compared to the presence of the two agents in separate cultures. The control containing dibutyryl cyclic AMP and SRBC produced fewer plaque-forming cells per culture than that containing only SRBC, but the responses were essentially the same when expressed as plaque-forming cells per 10^6 viable cells. The reduction in the plaque-forming cell responses due to the dibutyryl cyclic AMP alone, then, can be attributed to the smaller number of viable cells recovered after incubation. This latter observation is in agreement with a recent report¹⁶ that showed that cyclic AMP did not inhibit the *in vitro* immune response at concentrations used here. Repeated experiments showed the same pattern of dibutyryl cyclic AMP blockade of mitogen-induced suppressor activity. Further, dose-response studies with various concentrations of dibutyryl cyclic AMP showed that the concentrations (1×10^{-4} to 2×10^{-4} M) of the cyclic ribonucleotide that blocked the development of suppressor activity correlated with those concentrations that blocked the production of interferon in spleen cultures (Fig. 1) stimulated by the T-cell mitogens. Dibutyryl cyclic GMP, at the same concentrations, had no effect on either mitogen stimulation of interferon production or mitogen-induced suppression of the *in vitro* plaque-forming cell response.

Cholera toxin raises the endogenous level of cyclic AMP by stimulating adenyl cyclase activity¹⁷. The methyl xanthine 3-isobutyl-1-methyl xanthine (IMX) raises the endogenous cyclic AMP level by inhibiting phosphodiesterase activity¹⁸. Both agents blocked SEA suppression of the *in vitro* anti-SRBC plaque-forming cell response in a manner similar to that observed for dibutyryl cyclic AMP (Table 2). Further, they blocked SEA stimulation of interferon production in mouse spleen cell cultures at concentrations that blocked SEA suppressor activity. Theophylline, also a phosphodiesterase inhibitor, had an effect similar to that of IMX on the suppressor activity of SEA.

Dibutyryl cyclic GMP (10^{-3} and 5×10^{-4} M) did not affect the ability of dibutyryl cyclic AMP (1.4×10^{-4} M) to block the suppression of the plaque-forming cell response by SEA;

nor was the effect of dibutyryl cyclic AMP on mitogen stimulation of interferon affected by dibutyryl cyclic GMP. This is further evidence that the phenomena reported here are due to cyclic AMP, and are not influenced by cyclic GMP in this system.

The data are evidence that cyclic AMP, at the concentrations (2.0×10^{-5} – 2×10^{-4} M) employed here, selectively blocks mitogen induced suppressor cell activity, while not inhibiting T helper cell function, since SRBC is a thymus-dependent antigen¹⁴. Further, the biological function was associated with inhibition of mitogen induction of interferon in the spleen cell cultures. This suggests that mitogen-induced interferon is associated with mitogen-induced suppressor cell activity, and that the interferon may possibly be a mediator of such activity¹¹. In related studies, it has been shown that high concentrations (10^{-3} M) of dibutyryl cyclic AMP can inhibit the yield of interferon from phytohaemagglutinin-stimulated human peripheral leukocytes¹⁵ and from virus- or polyribonucleotide-stimulated mouse L cell cultures¹⁹. The data presented here show a logical and integrative relationship between cyclic AMP, lymphokine (interferon) activity, suppressor cell activity, and regulation of the immune response.

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Variant strain of rabbits lacking immunoglobulin κ polypeptide chain

The antibody molecule consists of heavy and light polypeptide chains¹. The light chains are shared by all immunoglobulin classes and they can be divided into two types, κ and λ . The great majority (up to 95%) of rabbit IgG molecules possess the κ type. This polypeptide chain, like many others, shows genetic polymorphism called allotypy²⁻⁸; the b locus of the rabbit determines allotypes which exist in four allelic forms, namely b4, 5, 6 and 9. They are inherited in Mendelian fashion as codominant genes^{3,4} and they have been correlated with amino acid sequences in the constant part of the κ chain, although some authors could associate amino acid differences in the variable part of this chain with certain b locus allotypes¹⁻⁷. The allotypes of the λ chain are determined by an independent c locus (specificities c7 and 21). They do not show Mendelian segregation, at least in our rabbit colony, although they do in certain strains^{4,8}.

We have established a new rabbit strain, named BASILEA, which derived from a mutant. Individuals of this strain lack the κ polypeptide chain which is replaced almost entirely by the λ type. The mutation, called *bas*, is an allelic form of the b locus.

A male rabbit (Fig. 1, no. III) was born in our colony. He lacked the b9 allotypic marker of the homozygous father (Fig. 1, no. I) who sired 35 normal offspring, all expressing this allotype. Rabbit no. III could have been classified as an illegitimate child. However, parents and offspring of this family have been checked for more than 20 genetic markers of immunoglobulins and all but one (b9) were in agreement with established patterns of inheritance. In addition, rabbit no. III has been tested with 15 anti-b9 antisera in double diffusion with negative results, although the method (see Fig. 2) is able to pick up antigen concentration as low as $10^{-1} \mu\text{g ml}^{-1}$.

Subsequently, rabbit no. III has been outbred and then back-crossed, and the progeny brother-sister mated. Table 1 summarises data from this breeding, and shows that this b allotype negative marker behaves like an allele at the b locus (for example, rabbit no. VI in Fig. 1).

Extensive immunisation³ with IgG *bas* of rabbits of various

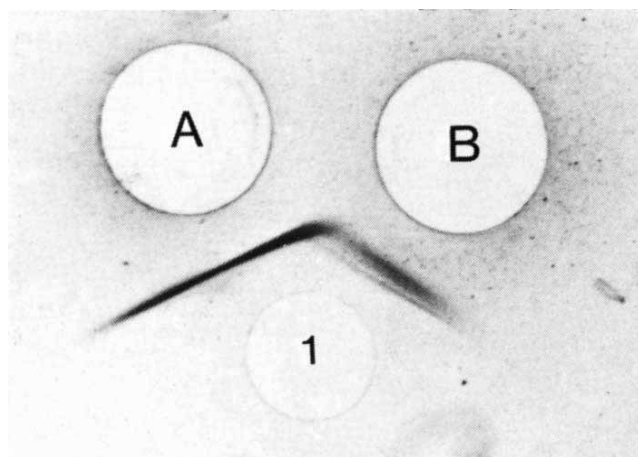


Fig. 2 Double diffusion pattern in agarose showing identity between anti-b9 produced in a standard rabbit (A) and in a *bas* mutant (B); (1) pool of IgG b9 (1 mg ml^{-1}). Double diffusion tests were performed in a gel composed of 1.5% agarose (A45, Indubiose, L'industrie Biologique Française, S.A.) and 2% polyethyleneglycol 6000 in PBS (phosphate buffered saline) at pH 7.2. The plates, 1 mm thick, with 5 mm distance between the wells, were developed at 4°C for 24–48 h. They have been examined unstained or stained with amino black or Coomassie blue. This method allowed us to see the precipitation lines at the antigen concentration as low as $10^{-1} \mu\text{g ml}^{-1}$.

genetic make up did not result in production of anti-allotypic antibodies against b *bas* as a whole. (It did, however, reveal another allotypic system which is the subject of a separate paper). On the other hand, homozygous *bas/bas* rabbits were capable of making good antibodies against the b4, 5, 6 and 9

Fig. 3 Direct binding of anti-light chain allotypic and anti-isotypic antisera to a pool of Fab *bas* fragment. Radioimmunoassay: Fab fragments of rabbit IgG have been prepared by papain digestion in a routine fashion and iodinated (^{125}I) using the chloramine T method ($50 \mu\text{g mCi}^{-1}$)⁹. The tests were performed immediately after labelling. The antigen was diluted in 1% ovalbumin made in PBS to give 10,000 c.p.m. per $50 \mu\text{l}$. This latter volume contained 8,000 c.p.m. of $^{22}\text{NaCl}$ as volume control. Various amounts of antiserum were mixed and incubated, 2–4 h at room temperature with this aliquot of antigen. After the incubation, 0.1 ml of 10% *Staphylococcus aureus* vaccine, strain Cowan I, has been added¹⁰. This strain is known to possess large amounts of a so-called protein A which binds Fc of most mammalian IgG classes. The *Staphylococcus* suspension was prepared in PBS containing 0.1% Na azide and 0.2% Nonidet P-40 detergent. This suspension was washed well to remove debris and other impurities of free protein A. After incubation the mixture was centrifuged at 5,000 r.p.m. for 15 min and about 50% of the supernatant removed, then the pellet fraction and the supernatant examined in the γ counter. Percentage binding, including volume control, was calculated according to the equation published previously¹¹. We controlled the maximum binding by titrating the antiserum and *Staphylococcus*. Non-specific binding was within the range of 3%. Tests were carried out in duplicate.

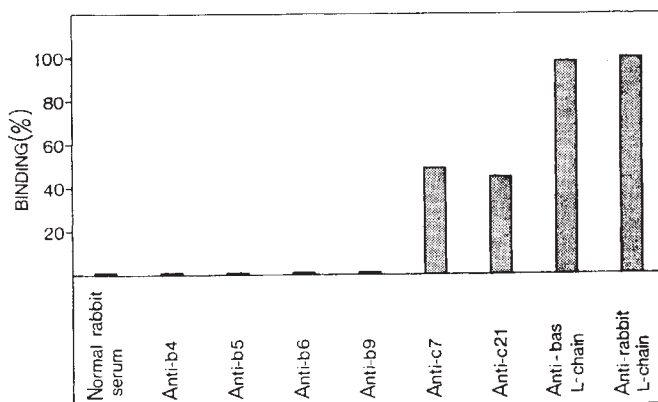


Fig. 1 Pedigree of the original *bas* family.

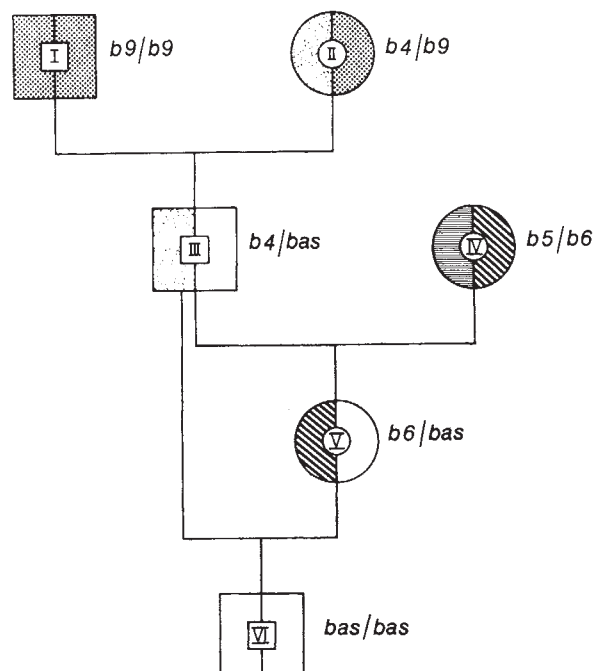


Table 1 Distribution of allotypes in the progeny of crosses involving the *bas* marker

Parents	Total	b allotype of progeny						χ^2	d.f.
			i	j	k	ik	jk		
<i>bas/bas</i> × <i>bas/bas</i>	16	16 (16)	—	—	—	—	—	—	—
<i>bas/bas</i> × <i>bⁱ/bas</i>	70	36 (35)	34 (35)	—	—	—	—	0.057	1
<i>bas/bas</i> × <i>b^j/bⁱ</i>	26	—	26 (26)	—	—	—	—	—	—
<i>bas/bas</i> × <i>b^k/bⁱ</i>	37	—	20 (18.5)	17 (18.5)	—	—	—	0.243	1
<i>b^k/bas</i> × <i>bⁱ/bas</i>	31	9 (7.75)	9 (7.75)	—	5 (7.75)	8 (7.75)	—	1.387	3
<i>b^k/bas</i> × <i>b^j/bas</i>	6	1 (3)	—	—	5 (3)	—	—	1.500*	1
<i>b^k/bas</i> × <i>b^k/b^k</i>	1	—	—	—	1 (1)	—	—	—	—
<i>b^k/bas</i> × <i>bⁱ/b^k</i>	6	—	1 (1.5)	—	1 (3)	4 (1.5)	—	3.417*	2
<i>b^k/bas</i> × <i>b^j/bⁱ</i>	47	—	23 (23.5)	—	—	24 (23.5)	—	0.021	1
<i>b^k/bas</i> × <i>b^k/b^j</i>	41	—	11 (10.25)	9 (10.25)	—	10 (10.25)	11 (10.25)	0.268	3
Total	281							6.893	12
								$P = 0.86$	

bⁱ, *b^j* and *b^k* represent various alleles at the *b* locus. For example, *bⁱ/b^j* are heterozygous and may be *bⁱ/b^s* or *b^j/b^s* or *b^s/b^s* . . . etc. Phenotypes *i*, *j* and *k* include also *bas* heterozygotes. The expected values, shown in brackets, are used for the χ^2 calculation.

*These values are calculated including the Yates' correction for continuity.

allotypes. It is worth noting that such an anti-b4 antiserum cross reacts strongly in double diffusion tests in gel, with b5 and b6. What is even more important, the *bas* rabbits can produce specific anti-b9 antiserum (see Fig. 2). This is convincing evidence that the ancestral b9 allotype was absent from the *bas* mutant.

To confirm the results obtained by double diffusion in gel that the κ chain allotypes were absent from IgG *bas*, direct binding of various allotypic and isotypic antisera to Fab *bas* was carried out. Figure 3 illustrates these results. None of the anti-b allotype antisera bound Fab *bas* in a detectable amount. On the contrary, anti-c7 and anti-c21 antisera, specific for λ chain allotypes, bound up to 95% of *bas* (50% and 45% respectively). Guinea pig anti-*bas* light chain and swine anti-rabbit light chain (Nordic Immunology) bound *bas* up to 100%. Anti- κ light chain antisera were excluded from this test as non-specific (contaminated with anti- λ antibodies).

Guinea pigs were immunised with λ light chain from rabbits suppressed for the *b* locus allotype¹²⁻¹⁴, and with the *bas* light chain. Figure 4 illustrates that the *bas* chain is either identical with or very closely related to the light chain of the suppressed rabbits.

This observation has also been confirmed by the inhibition of direct binding of anti-*bas* light chain to Fab *bas* (Fig. 5). Fab fragments were used as inhibitors (light chain preparations gave similar results). The homologous Fab fragment (*bas*) inhibited the binding 100%, whereas the Fab of suppressed rabbits showed biphasic curves reaching also almost 100% inhibition eventually. Altogether only 50% of this light chain (as Fab) binds anti-c7 and anti-c21 antibodies, and therefore may contain determinants in a concentration different from that of the homologous Fab

bas. When Fab b9, which contained 10% non-b9 (λ chain), was used as inhibitor, a pattern similar to the homologous one was observed but the curve was shifted by one order of magnitude. The b9 preparation came from rabbits closely related to the strain BASILEA. The controls with Fab b4 and b5 showed different inhibition curves, indicating much less similarity between *bas* and b4 or b5. This can be explained by the presence of λ chain in these preparations. Nevertheless, weak cross-reactivity between κ and λ chains cannot be ruled out. The radioimmunoassay revealed in addition that the preparations of the *bas* chain and of the light chain of suppressed rabbits has about 5% of either subtype of κ or subtype of λ ¹⁵.

Preliminary immunochemical data (in collaboration with Dr J-C. Jaton) show that the amino acid composition of the *bas* light chain resembles the λ type. As judged by similarities, the peptide maps of the light chains^{16,17} fall into two groups; group I: b9, *bas* light chain and that of suppressed rabbits (almost entirely λ), group II: b4, b5 and b6 (all three chain preparations almost entirely of the κ type).

To see how the *bas* mutation influences the expression of the *b* locus, various Fab preparations from rabbits, homozygous or heterozygous for the *b* locus were tested for binding to anti-b allotype sera. The antisera used were strictly specific; none of them bound Fab *bas* but they reacted with 90-95% of their corresponding Fab. The same was true when Fab preparations from animals heterozygous for *bas* and the corresponding *b* allele were tested. Fab b4-5 from heterozygotes show the

Fig. 5 Inhibition of binding of anti-*bas* antiserum to Fab *bas* by various Fab fragments. The test was performed as described in Fig. 3 but different amounts of inhibitor were added to the antigen before application of the antiserum. A serum concentration was used to get 50% binding without inhibitor. Percentage inhibition was calculated according to a published formula¹¹. Each point represents the mean of quadruplicates. ●, *bas*; ◆, suppressed for b5; ○, b9; ◇, b5; ▲, b4.

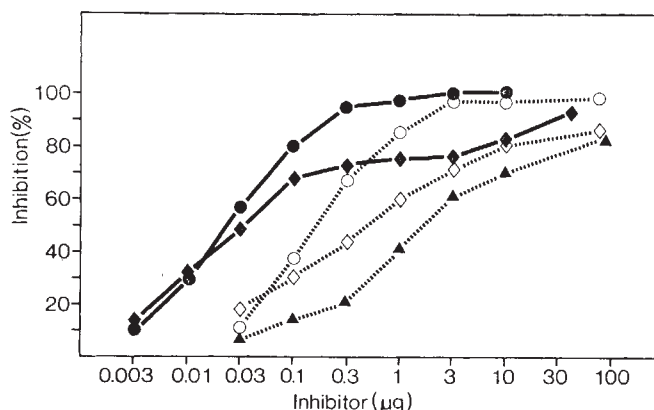
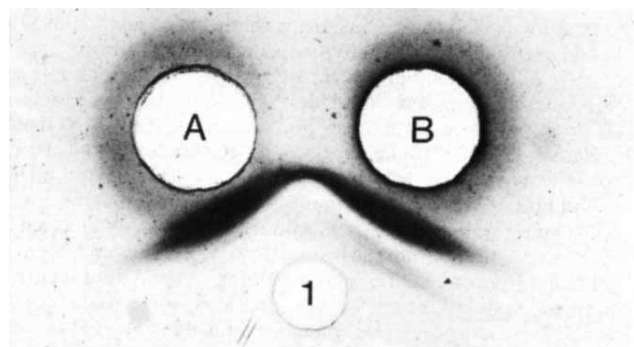


Fig. 4 Double diffusion pattern in agarose showing identity or close similarity between anti-*bas* light chain (A) and anti-light chain from suppressed rabbits (B) made in guinea pig; (1) light chain from rabbits suppressed for the *b*-locus 1 mg ml⁻¹. The test was performed as described in Fig. 2. The precipitation was overdeveloped to see also minor components. When *bas* light chain was used as antigen analogous results were obtained.



expression of both alleles as expected⁴. By analogy, with the rabbits heterozygous at the b locus and suppressed¹⁴ for one allele only, the heterozygotes *b4/kas* and so on maintain a normal κ : λ ratio, that is, one ' κ chromosome' is enough to regulate this ratio.

Rabbits of the strain BASILEA are healthy and have normal level of IgG unlike the few cases described in man^{18,19}. They produce good antibodies to rabbit allotypes and idiotypes, against sheep erythrocytes, dinitrophenol (DNP), and *Proteus*, *Pneumococcus* and *Streptococcus* vaccines. Immunisation with the group A-variant streptococcal vaccine (in collaboration with Dr D. G. Braun) gave a high proportion (8 out of 10 individuals) of restricted immune response²⁰ as judged by microzone electrophoresis and isoelectric focusing. The antibodies eluted from the bacterial cell wall and tested serologically proved to be of the λ type.

In conclusion, we may say that the *bas* variant of the rabbit arose by spontaneous mutation affecting the b locus of the chromosome region coding for the κ polypeptide chain or an essential part of it. Deletion seems to be the most likely mechanism, although many other explanations could be considered. The BASILEA strain resembles rabbits suppressed completely for the b locus allotypes, and it offers immense experimental opportunities.

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Lymphocytes with T-cell markers cooperate with IgG antibodies in the lysis of human tumour cells

THYMUS-DERIVED (T) lymphocytes from animals immunised with syngeneic or allogeneic tumour cells are well known to kill specifically target cells *in vitro*¹. The as yet unidentified antigen recognition structure on T lymphocytes is not deemed to be a conventional type of immunoglobulin. In contrast to this kind of cell-mediated cytotoxicity another cellular killing mechanism has been described, called antibody-dependent cell-mediated cytotoxicity (ADCC), in which cells bearing the Fc receptor (FcR) cooperate with humoral antibody in the cytolytic action^{2,3}. The nature of the main lymphoid effector cells (K cells) involved in the latter system is still ill-defined. They are supposed not to be T lymphocytes. Recently however, Lamon *et al.* reported on mouse thymocytes which were able to lyse MSV-induced tumour cells using IgM

antibodies⁴. In this report we present evidence that human lymphocytes bearing the T marker in collaboration with homologous or autologous antibodies can efficiently kill tumour cells *in vitro*.

The data were obtained with an ADCC system^{5,6} consisting of cultured melanoma cells as target cells, different preparations of human peripheral blood lymphocytes as effector cells, and anti-melanoma cell antisera obtained from melanoma patients. The cytolytic activity of lymphocytes was measured by the ³H-proline release test as described previously⁷. Antibodies increasing the spontaneous cell-mediated cytotoxicity (CMC) of melanoma cells by more than 100% were detected in 17 out of 87 patients. Immunoglobulins were isolated from patients' sera by affinity chromatography using insolubilised antibodies rendered monospecific for heavy chain determinants of either IgG, IgM or IgA. Non-adherent lymphocytes were prepared as described previously⁷. T lymphocytes defined as cells having receptors for sheep erythrocytes (SRBC), were purified by two different procedures: either by passing the cells through IgG anti-IgG Degalan⁸-bead columns according to Golstein *et al.*⁸ or by density gradient centrifugation as spontaneous rosettes with SRBC (E-RFC)⁵. The various lymphocyte preparations were characterised by surface markers as depicted in Table 1.

As can be seen from Fig. 1a the passage of lymphocytes through an IgG anti-IgG bead column resulted in a complete loss of spontaneous cytotoxicity. However, the capacity of column-filtrated cells to cooperate with anti-tumour antibodies was not affected when tested in the long term ³H-proline release assay. Furthermore, these cells still responded to mitogens such as PHA and concanavalin A indicating functional integrity (Table 1). When—as a control—cells were passed through a FCS-coated Degalan column, neither the CMC- nor the ADCC-activity was reduced. These findings obtained in eight independent experiments were rather unexpected since almost no FcR-bearing cells could be detected immediately after column fractionation when tested by a sensitive radioautographic method using ¹²⁵I-labelled aggregated IgG (¹²⁵I-agg. IgG) of defined size⁹ (Table 1).

Because ADCC is thought to depend on the presence of FcR-bearing cells the question arose whether Fc receptors and ADCC activity were generated *de novo* during the 40 h cocultivation with target cells. Therefore, column fractionated lymphocytes were incubated together with unlabelled tumour cells for 6, 12, and 48 h. After various periods of incubation the FcR-bearing cells were enumerated and ADCC was assessed in a short-term 6 h ³H-proline release assay. As demonstrated in Table 2 the percentage of FcR-bearing cells increased with time of cocultivation, reaching approximately the pre-filtration value after 48 h. No ADCC activity could be detected immediately after the column passage, independent of the effector cell–target cell ratio used (Fig. 1b). However, significant ADCC reappeared after 12 to 24 h of cocultivation with either syngeneic or allogeneic melanoma cells, and increased further up to 48 h. In some experiments (for example experiment 2, Table 2) the generated ADCC activity considerably exceeded the prefiltration value. In control experiments where lymphocytes were cultured without tumour cells under otherwise identical conditions very few FcR-bearing cells could be detected up to 48 h and ADCC remained marginal when compared with that of cocultured cells. Thus, the increase of the number of FcR-bearing cells and ADCC activity during cocultivation may not be due to deblocking of FcRs occupied by IgG or complexes released from the column. This assumption is supported by additional observations (data not shown): first, when ¹²⁵I-labelled goat anti-human IgG or ¹²⁵I-labelled human IgG (at least 100 μ Ci per column) were used for the preparation of the IgG anti-IgG column, neither by direct counting in a gamma counter nor by radio-autography could a significant radioactivity be detected on the passed cells. Second, the proportion of FcR-bearing cells after column filtration did not increase after trypsin treatment. In addition, unspecific retention of cytotoxic effector cells on protein-coated Degalan beads was rendered unlikely, since FCS-coated control columns did not reduce the proportion of FcR-bearing cells. The difference in cell yield, however, between both columns

Table 1 Characterisation of purified lymphocytes immediately after filtration through IgG anti-IgG bead columns or FCS-coated control columns and separation of E-rosette forming cells

Lymphocyte preparation	FITC-anti-Ig-positive cells (%)	¹²⁵ I-aggregated IgG-binding cells (%)	¹²⁵ I-E-rosette forming cells (%)	monocytes* (%)	³ H-thymidine incorporation (c.p.m.) at optimal concentrations of PHA (10 µg ml ⁻¹) Con A (25 µg ml ⁻¹)	
Non-adherent lymphocytes	5.2 ± 1.7	10.0 ± 2.8	83.0 ± 5.5	1.9 ± 0.6	53,840 ± 1924	26,920 ± 1793
IgG anti-IgG† column filtrated lymphocytes	<0.1	<0.1	96.2 ± 3.1	<0.2	17,498 ± 1302	6,730 ± 91
FCS-column‡ filtrated lymphocytes	—	8.1 ± 2.4	80.3 ± 2.1	—	—	—
Pelleted E-rosette forming cells	0.5 ± 0.2	3.5 ± 0.7	96.8 ± 2.7	<0.2	20,190 ± 1744	15,479 ± 998

Results are means ± s.d. of five independent experiments, except the PHA and con A stimulation where the data of one representative experiment are given. Preparation of effector cells from human peripheral blood and determination of surface markers was performed as described previously^{5,7,9}. SRBC were lysed by short (20–40 s) hypotonic treatment with distilled water. ³H-thymidine incorporation in con A and PHA-stimulated cultures was determined using 1 × 10⁶ lymphocytes, cultured in 1 ml RPMI 1640 supplemented with 10% human AB-serum for 48 h in the presence of various concentrations of PHA-P (DIFCO Laboratories) (1–10 µg ml⁻¹) and con A (Pharmacia Fine Chemicals) (0.1–25 µg ml⁻¹). After 40 h of culture 1 µCi ml⁻¹ ³H-thymidine was added for an additional 8 h. Results are expressed as mean c.p.m. of triplicate cultures ± s.e.

* Percentage of monocytes was estimated according to reactions for N-AS-Acetate-Esterase and Peroxidase.

† Recovery of cells from the column: 50–60%.

‡ Recovery of cells from the column: 80–85%.

** Radioactivity incorporated in unstimulated cultures 673 ± 312 c.p.m.

cannot be fully explained by retention of cells bearing detectable FcRs and/or S-Ig (Table 1).

More than 95% of the column fractionated cells proved to be E-RFCs. Since a considerable portion of the FcR-bearing lymphocytes generated *in vitro* also bound SRBC, it was assumed that these cells were derived from the original T-cell inoculum. Such double marker cells are also present in the peripheral blood as originally described by Dickler *et al.*¹⁰ Thus lymphocytes were isolated as E-rosettes and found to contain about 3.5% of ¹²⁵I-agg. IgG-binding cells, most of them simultaneously binding SRBC (Table 1). These purified E-RFCs displayed ADCC activity comparable with unseparated lymphocytes in a 6 h ³H-proline release assay without prior cultivation on tumour cells (Fig. 1c).

The antibodies which induce cytolytic effects with the subset of lymphocytes described above could be confined to the IgG class as demonstrated in Table 3. IgM or IgA fractions of the antisera tested so far were not active in ADCC.

If the binding of SRBC is considered a valid marker for human T lymphocytes, the data presented strongly suggest that a sub-

population of T lymphocytes is able to induce lysis of tumour cells with the help of IgG antibodies. So far, only peripheral T lymphocytes showed this effect, whereas thymocytes were inactive. The mechanism of the *in vitro* induction of cooperative cytotoxicity is unknown. The time kinetics suggest that activation by lymphocyte–tumour cell contact may lead to a rapid reappearance of Fc-receptor activity on the effector cells, which in turn might increase ADCC activity. In agreement with the data presented are the observations of Biberfeld and Perlmann¹¹ describing a considerable proportion of lymphocytes bearing T-cell marker in a cytolytic plaque assay. Receptors for the Fc-portion of either the IgG^{12–15} or the IgM^{4,15} molecule have been described for various T-cell subpopulations. The finding that spontaneous cytolytic activity is lost by passage of the lymphocytes through IgG-anti-IgG columns, also described by others¹⁶, suggests that FcR- and/or S-Ig bearing cells are effector cells in spontaneous cytotoxicity. Recent findings in our laboratory (J. G. Saal *et al.*, in preparation) together with the data presented here, point to the possibility that T lymphocytes armed *in vivo* by humoral IgG

Table 2 Appearance of aggregated IgG binding and ADCC activity during cocultivation of human lymphocytes bearing T markers with allogeneic and syngeneic melanoma cells

Expt	Cultivation of purified lymphocytes	%CTL in a 6-h ADCC assay			Before column filtration	% ¹²⁵ I-agg. IgG-binding cells				
		Before column filtration	Period of cultivation after column filtration (h)			Before column filtration	Period of cultivation after column filtration (h)			
			0	12			48	0	12	24
1	With allogeneic tumour cells	17 ± 4	0 ± 0	4 ± 1	5.5	0.2	0.8	1.5	2.5	
	12 ± 1			0.3			0.1	0.2		
2	Without tumour cells	13 ± 1	0 ± 0	0 ± 0	5.5	0.1	3.2	—	5.0	
	3 ± 2			8 ± 3			0.1	—	0.1	
3	With allogeneic tumour cells	10 ± 1	2 ± 1	8 ± 1	6.7	0.5	1.4	1.8	4.7	
	53 ± 2			0.3			0.2	0.3		
	Without tumour cells			6 ± 2						
				1 ± 1						
				2 ± 1						

Lymphocytes from melanoma patients were depleted for Fc-receptor- and S-Ig-bearing cells by filtration through IgG anti-IgG bead columns and cultivated either alone or together with 1 × 10⁴ syngeneic or allogeneic melanoma tumour cells in Falcon-Microtest II plates. The cells were collected at the intervals indicated and tested for either ADCC activity in a 6-h ³H-proline release test (as described in legend to Table 3) or uptake of ¹²⁵I-agg.-IgG by radioautography. Effector cell–target cell ratio = 50:1.

Fig. 1 Spontaneous (CMC) and antibody dependent (ADCC) cytolytic activity of different lymphocyte preparations. Open circles (○) indicate the cytolytic activity of cells before column filtration or E-rosette centrifugation (non-adherent lymphocytes). ●, Cells after passage through IgG anti-IgG columns; ▲, cells after passage through FCS-coated control columns; ■, pelleted E-RFCs. CMC is given by the dashed lines and ADCC by the solid lines. Cytolytic activities were measured either in a 6-h (expt. b, c) or a 40-h (expt. a) ^3H -proline release test. The test was performed as described in legend to Table 3. Effector cell-target cell ratios of 25/1, 50/1 and 100/1 were tested. Results are means of triplicate tests $\pm$ s.e. In the 6-h assays depicted in Fig. 1b, c no measurable CMC was detected.

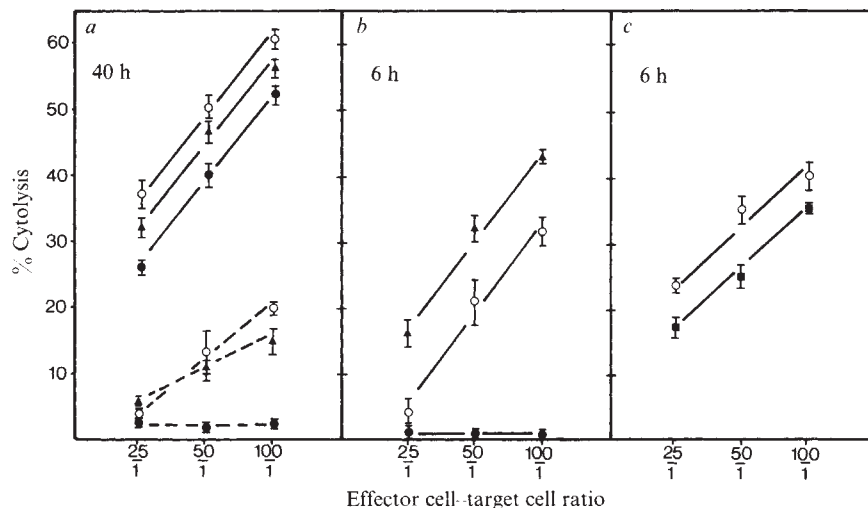


Table 3 Cytolytic activity of lymphocytes before and after filtration through IgG anti-IgG bead columns: cooperative effect with antibodies directed against melanoma cell membrane

Antiserum added	Cytolysis (%) in a 40-h ^3H -proline release test with	
	Non-adherent lymphocytes	Lymphocytes after IgG anti-IgG column fractionation
No serum (CMC)	17 $\pm$ 1	0 $\pm$ 0
Whole serum	44 $\pm$ 1	40 $\pm$ 1
IgG fraction	39 $\pm$ 1	39 $\pm$ 1
IgM fraction	14 $\pm$ 2	2 $\pm$ 1
IgA fraction	11 $\pm$ 3	n.t.

CMC was measured by the ^3H -proline release test as previously described in detail⁷. $5 \times 10^6 - 1 \times 10^7$ mel-Ei-78 tumour cells labelled 24 h with $50 \mu\text{Ci } ^3\text{H}$ -proline ($500-1,000 \text{ mCi mmol}^{-1}$) were distributed into Falcon Microtest-II plates (1×10^4 per well). 5×10^5 effector lymphocytes were added in RPMI 1640 with 10% human AB serum. ADCC was assessed by adding a human anti-melanoma antiserum to the CMC system at a final dilution of 1:6. IgM or IgA was isolated from the antiserum by affinity chromatography with sepharose-coupled antibody previously rendered monospecific for the IgG-IgM- or IgA-heavy chains.

ADCC is defined as antibody-induced cytotoxicity superimposed on spontaneous CMC. The antiserum without lymphocytes never showed more than 4% cytotoxicity (CTL). After 6-40 h of incubation at 37°C the total content of each well was collected on glass filters by use of an automatic harvesting device. The radioactivity retained on the filter was determined in a liquid scintillation counter.

CTL was calculated by:

$$\% \text{CTL} = 100 - \frac{\text{c.p.m. test sample}}{\text{c.p.m. medium control}} \times 100$$

Results are means of triplicate tests $\pm$ s.e.
n.t., not tested.

antibodies are involved in spontaneous cytotoxicity.

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Cμ4 domain of IgM has cytophilic activity for human lymphocytes

WE have isolated and identified two intact Cμ4-domain fragments from a monoclonal IgM protein. They differed only in their carbohydrate content, having the same polypeptide chain. They were separated from each other and from Fcμ monomer by molecular exclusion and affinity chromatography (insolubilised concanavalin A¹) of mildly reduced and alkylated Fcμ produced by the method of Plaut and Tomasi². The Cμ4 fragment containing carbohydrate was designated Cμ4(+) while the carbohydrate-poor domain was called Cμ4(-). These two fragments were identified as Cμ4-domains from their amino acid sequences, amino acid compositions, molecular weights (14,700) and antigenic characteristics. Both fragments originate from tryptic cleavage at Lys-445 of the μ chain (IgM(Ou) numbering³) and could not be further fragmented by extensive reduction and alkylation⁴. The domain hypothesis of Edelman *et al.*⁵ proposes that each homology region of the immunoglobulins evolved to perform independent biological effector functions. Hurst *et al.*⁶ have shown that fragmented Cμ4 domain will fix activated Cl(CI) and will also activate proenzyme Cl to Cl⁷. Moretta *et al.*⁸ have demonstrated receptors specific for IgM on human T lymphocytes, and this has been confirmed by McConnell and Hurd⁹. We now report that these receptors are specific for the Fc part of IgM (Table 1) and furthermore that the Cμ4 domain is involved in this affinity reaction in a dose-dependent manner (Fig. 1).

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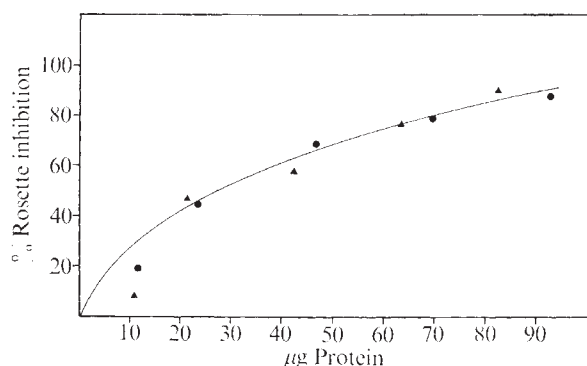


Fig. 1 Dose-dependent inhibition of EA(IgM) rosette formation by C4(+) protein. Methods used were the same as that described in Table 1. ●, Experiment 1; ▲, experiment 2.

Several workers have concluded that the classical pathway of complement activation is mediated by the Cγ2 (ref. 13) region of IgG (Table V, ref. 14). In the case of cytophilic reactions, however, the situation is not so well defined; Cγ2, Cγ3 or intact Fc region being implicated (Table V, ref. 14). Our results show that Fcμ (that is Cμ3-Cμ4) and Cμ4 fragment inhibit EA(IgM) rosette formation to approximately the same extent, and it must therefore be deduced that the Cμ3 domain does not contribute significantly to this activity. In contrast to Fcγ, minimal non-covalent interactions occur in Fcμ (ref. 15), and as a result the protein exists mainly as a monomer in solution. This is important because both Fcμ and Cμ4 inhibit rosette formation quite efficiently (Table 1), indicating that the Fcμ

Table 1 Inhibition of EA(IgM) rosette formation by IgM and some of its fragments

Inhibitor	Concentration (mg ml ⁻¹)	Inhibition of EA(IgM) rosettes (%) exp. 1	exp. 2
None	—	0	0
IgG	9.9	2.2	0
IgM	0.52	81.9	90.6
Fc5μ	0.50	97.8	96.1
Fcμ	0.52	53.6	58.6
Fabμ	4.1	0	0
Cμ4(+)	0.42	47.8	46.1
Cμ4(-)	0.70	65.2	59.4

The formation (and inhibition) of IgM-dependent rosettes (EA(IgM)-RF) was carried out as described by Moretta *et al.*⁸ Rabbit antisera containing mainly IgM antibodies to ox erythrocytes were raised according to Mayer¹⁰ and the fraction containing IgM was isolated by molecular exclusion chromatography on Sephadex G-200. IgG was purified by DEAE-cellulose chromatography of Cohn fraction II and IgM, Fc5μ, Fcμ and Fabμ as previously described for normal IgM¹¹. The protein concentration of the inhibitors tested was determined by the Folin Ciocalteu method¹² using recrystallised bovine serum albumin as standard. Three hundred lymphocytes were counted for each test and only those cells with three or more adhering EA(IgM) were scored as rosettes.

receptor does not require antigen binding to or a polymeric form of IgM in order to fulfil its unknown function. Two biological functions—complement fixation⁹ and cytophilic activity—have now been attributed to the Cμ4 domain of IgM.

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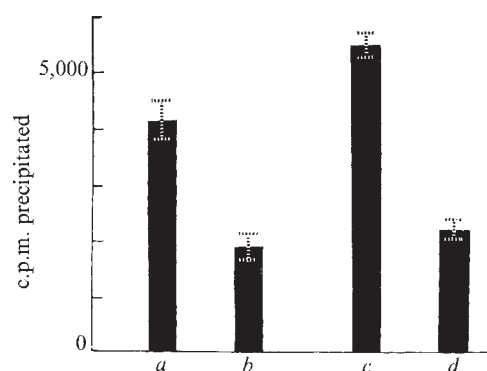
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Common antigens and male-female recognition in plants

THE question of how the female stigma of the plant recognises a pollen grain carrying a compatible male gamete from all the possibilities presented to it by air currents and visiting insects is an intriguing problem¹. When pollen is recognised at the stigma surface, a mixture of components, including proteins and glycoproteins, are released from cavities in the pollen walls^{2,3}. These components have been partially characterised^{4,5} and include those essential to the recognition process⁶. The pollen wall components flow over and bind to the stigma surface which is covered by a secreted sticky coating⁷. The binding of specific recognition factors determines the success of a mating⁸. We have attempted to identify factors involved in pollen-stigma recognition, using immunochemical methods with rabbit antisera to pollen and stigma surface components. The major antigen of the stigma surface shows immunological identity and has the same molecular weight as an antigenic pollen wall component. This stigma antigen is also partially identical with components of other tissues of the same plant. Absorption experiments show that the cross-reacting antibodies can be removed from the antisera, leaving antibodies which interact only with the immunising antigen. This raises the question whether plant cells, like animal cells⁹, are specified according to their family, species, organ and tissue by surface determinants.

Antisera were raised in rabbits to both stigma surface extracts and pollen wall components of *Gladiolus*

Fig. 1 Immune precipitation of ¹²⁵I-pollen and stigma antigens of *Gladiolus* by homologous and heterologous antisera. A double antibody precipitating system was used: to 200 μl of radioiodinated pollen or stigma extracts (10⁶ c.p.m.) were added 100 μl of a 1 : 20 dilution of rabbit antiserum. The precipitate was developed by addition of 200 μl of a 1 to 4 dilution of goat antiserum to rabbit IgG followed by incubation for 1 h at 37 °C and 15 h at 4 °C. Data given are ¹²⁵I-radioactivity associated with precipitates which were washed twice by centrifugation. a, ¹²⁵I-pollen + anti-pollen serum; b, ¹²⁵I-pollen + anti-stigma serum; c, ¹²⁵I-stigma + anti-stigma serum; d, ¹²⁵I-stigma + anti-pollen serum.



gandavensis cv¹⁰. Stigma antigens were prepared by washing receptive stigmas with 0.05 M Tris buffer, pH 8.4, 0.15 M NaCl and 0.001 M CaCl₂, and dialysing and freeze drying the material washed from the surface. Pollen antigens were allowed to diffuse from the wall cavities for 10 min in the same buffer and the filtrate was dialysed and freeze dried.

Samples of both stigma and pollen antigens were labelled with ¹²⁵I by the lactoperoxidase method⁹ and incubated with both the homologous and heterologous antisera. The immune precipitates were collected, washed and counted. The results (Fig. 1) show specificity of precipitation in both the homologous stigma-antistigma and the pollen-antipollen systems, as well as some cross reactivity in the heterologous systems.

The washed immune precipitates were dissociated and the components were separated by sodium dodecyl sulphate (SDS) polyacrylamide gel electrophoresis. The gels were cut into 2-mm slices which were counted individually. The distribution of radioactivity is shown in Fig. 2. These patterns show a major antigen in the stigma surface preparation, molecular weight ~40,000, and this cross reacts with antisera raised to pollen from the same plant. The reciprocal experiment confirms this: pollen contains seven major antigens, of which that of molecular weight ~40,000 cross reacts with antisera raised to the stigma surface.

In gel immunodiffusion experiments (Fig. 3), antisera to stigma surfaces show two bands, one of which shows partial identity with a petal component and a pollen wall component. Of the five precipitating antigens of pollen, two are common to both corm and petal, and the slower diffusing of these is also common with the stigma. In addition, petal also contains two other components which form precipitin bands with antisera to pollen, and one of these is also present in corm.

Absorption of antisera to stigma surfaces with petal

Fig. 2 Analysis of ¹²⁵I-stigma (a) and pollen (b) antigens of *Gladiolus* on SDS polyacrylamide gels after precipitation of homologous antisera (solid line) and heterologous antisera (broken line). Immunoprecipitates were dissociated in 200 µl of Tris buffer, pH 6.8, containing 3% SDS and 5% β-mercaptoethanol, and run on SDS polyacrylamide gels as described by Laemli and Favre¹⁶. The gels were sliced and radioactivity was determined using a Packard autogamma counter.

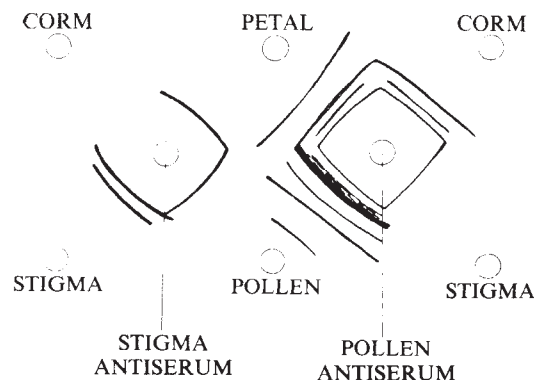
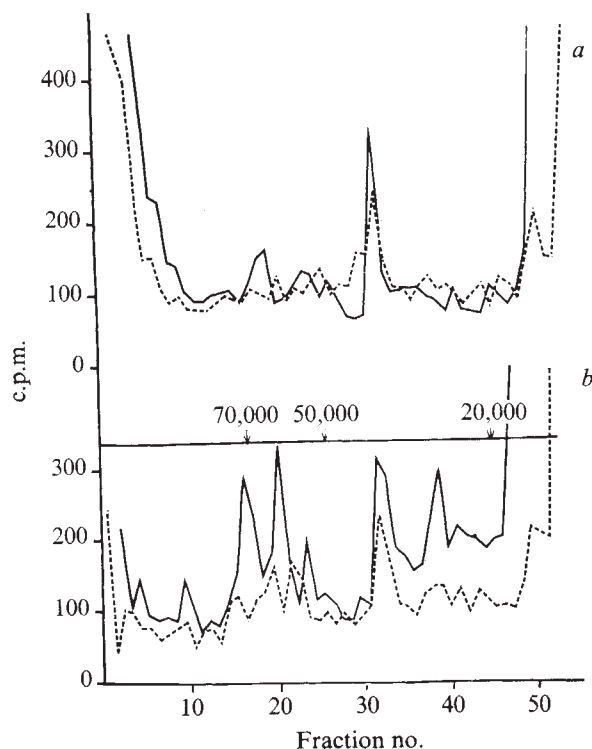


Fig. 3 Immunodiffusion of *Gladiolus* tissue antigens against antisera raised to stigma surface preparations and pollen wall components. Agar gel immunodiffusion plates were run using antigens at 10 µg ml⁻¹ in 0.15 M NaCl and whole antisera. Antigens were prepared by homogenising the tissue (0.25 g fresh weight per ml of 0.05 M Tris, pH 8.5, 0.15 M NaCl, 0.001 M CaCl₂) and dialysing and freeze drying the supernatant.

antigen removed the capacity of the serum to precipitate the petal antigen, but did not apparently affect the capacity of the serum to precipitate either the pollen or the stigma antigens. Likewise, the absorption of the antisera with pollen antigen removed the capacity of the serum to precipitate the pollen antigen, but the absorbed antisera retained the capacity to precipitate the stigma antigens. Stigma-absorbed antisera would not precipitate with either petal, pollen or stigma antigens.

One explanation for these results is that stigma surface determinants in this system are partially identical with determinants of other tissues of the same plant. It may be that variation in the degree of identity of this component in pollen and stigma controls the positive recognition in the pollen-stigma system. This conforms with suggestions¹²⁻¹⁴ that the recognition genes in self-incompatible plants may specify identical products in stigma and pollen. Several groups of stigma surface components have been described⁸, but their individual antigenicity is not known. It is, however, possible that some of the antigens are isozymes of hydrolytic enzymes common to both sites¹⁵.

The finding that a closely related determinant is present in petals, and the observation of antigens common to pollen and other tissues of the same plant, may indicate that male-female recognition factors are part of a wider system of recognition factors involved in such basic functions as cell growth and differentiation, which are reflected in economically important problems such as scion-rootstock incompatibility in fruit trees and other horticultural crops.

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Solubilisation of phospholipid membranes by human plasma high density lipoproteins

HUMAN plasma high density lipoproteins (HDL) seem to have a protective effect against the development of atherosclerosis^{1,2}. Glomset proposed that unesterified cholesterol diffuses from cell membranes into the HDL particle following esterification of cholesterol in HDL by the action of plasma lecithin: cholesterol acyltransferase³. In tissue culture experiments, HDL⁴ and complexes of its apoproteins with phospholipids⁵ removed cholesterol from arterial smooth muscle cells, and the principal HDL apoprotein, apo-A1, adsorbed to and removed phospholipid from Land-schutz ascites cells⁶. We show that intact HDL releases part of its apoprotein when incubated with synthetic phospholipid membranes, leading to the formation of soluble, discoidal phospholipid-apoprotein complexes. The residual apoprotein-deficient HDL particles seem to undergo fusion.

The human plasma HDL are spherical particles, 95-105 Å in diameter, consisting of a polar shell of phospholipid head groups and apoproteins surrounding a core of apolar lipids^{7,8}. The protein moiety comprises about 60% apoprotein-A1 (molecular weight 28,331)⁹ and 30% apoprotein-A2 (molecular weight 17,380)¹⁰. Studies of HDL by differential scanning calorimetry have shown that apo-A1 is released from HDL following heating to 100 °C, giving rise to a characteristic reversible thermal denaturation of lipid-free apo-A1^{11,12}. This transition with midpoint temperature at 54 °C has been used to monitor release of apo-A1 from HDL (Fig. 1). By heating HDL, cooling, then reheating to progressively higher temperatures, the fraction of apo-A1 released with each increment in temperature was calculated from the enthalpy of the transition at 54 °C. These experiments indicate that almost all of the apo-A1 of each HDL preparation can be released in lipid-free form between 55 and 80 °C. Between 80 and 95 °C cholesterol ester is liberated from HDL, which can be demonstrated on cooling by the appearance of reversible liquid crystalline transitions between 11 and 33 °C (Fig 1g), and the presence of smectic liquid crystals by polarising microscopy¹³. With each HDL preparation more than 90% of the apo-A1 was released before cholesterol ester liquid crystalline transitions could be demonstrated. Since domains of cholesterol ester as small as 1,000 molecules (as in low density lipoprotein) show liquid crystalline transitions¹⁴, HDL partially depleted of apo-A1 must exist as a stable microemulsion containing small domains of cholesterol ester, probably fewer than 1,000 molecules.

Apo-A1, released from HDL by heating, could be isolated by preparative ultracentrifugation. HDL was heated to 70 °C for 15 min, cooled and centrifuged at d (density) 1.25 g ml⁻¹.

The infranatant contained 5-15 times more lipid-free (that is, containing less than 1% phospholipid) protein than control, unheated HDL preparations, and when examined by Sephadex G-200 chromatography and polyacrylamide gel electrophoresis, the protein consisted of >90% apo-A1. The

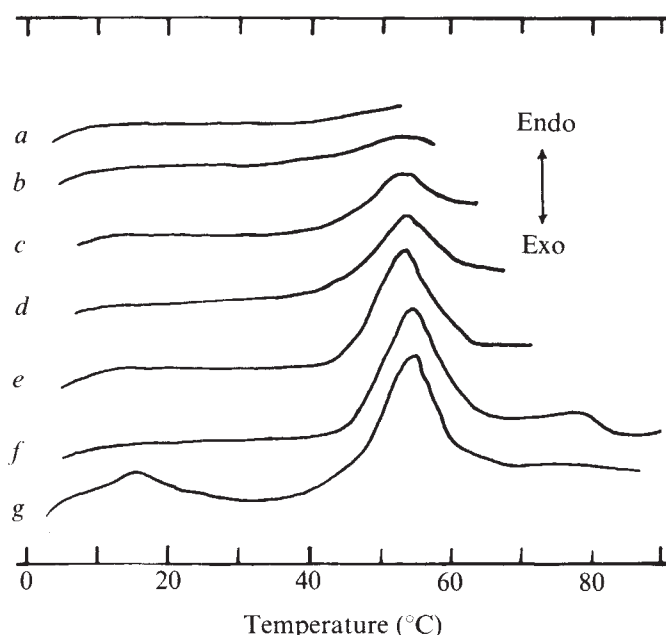


Fig. 1 Differential scanning calorimetry tracings of the thermal disruption of HDL. Human plasma HDL was isolated from the blood of normal fasting male donors by ultracentrifugal flotation between 1.063 and 1.21 g ml⁻¹ as previously described¹². The lipoprotein samples were concentrated to 10-30% solids by vacuum dialysis. Differential scanning calorimetry experiments were performed on a Perkin Elmer DSC-2B, calibrated with cyclohexane and indium. Experiments were performed in 75 µl sealed stainless steel pans at heating rates of 2.5 or 5 °C min⁻¹. HDL was heated to progressively higher temperatures with 5 °C increments on each heating run, and cooled at 10 °C min⁻¹. The heating runs to 75, 80 and 85 °C were identical to e. Experimental results were independent of lipoprotein concentration. In experiments where apo-A1 was heated repeatedly to the same temperature, above 55 °C, there was an increase in the endotherm at 54 °C, on the second heating run (by 10-40%), the third heating run (5-10%), but not on the fourth and subsequent heating runs. Repeated heating to temperatures below 55 °C did not result in liberation of apo-A1. The low temperature transition (Fig. 1g) is attributed to cholesterol ester because it has the same temperature and enthalpy as the smectic-liquid transition of cholesterol ester isolated from HDL, and the same temperature as the smectic-liquid transition of cholesterol ester observed by polarising microscopy of heat disrupted HDL. Further, a similar transition is not observed in any of the other lipid or protein components of HDL.

supernatant was optically clear and by electron microscopy (EM) contained spherical particles of diameters 90-180 Å (mean ± s.e.m. = 145 ± 3.1 Å, n=90), which were significantly larger than those present in control preparations (91 ± 2.8 Å (n=100)). The larger particles present in the supernatant probably arose from fusion of HDL particles secondary to the loss of amphiphilic apoprotein molecules. In experiments where HDL was heated to a series of temperatures above 55 °C, an increasing amount of pure apo-A1 was recovered in the infranatant between 60 and 85 °C, in quantities corresponding to the increasing enthalpy of the 54 °C transition in the differential scanning calorimetry experiments (Fig. 1).

To determine if apo-A1 could be removed from HDL at 37 °C, we incubated intact HDL with turbid suspensions of a synthetic phospholipid, dimyristoyl lecithin (DML). There was clearing of phospholipid turbidity (measured by the decrease in optical density at 640 nm), indicating dissolution of multilamellar phospholipid liposomes. Differential scanning calorimetry tracings of incubated mixtures of HDL and DML are shown in Fig. 2. The addition of HDL to DML resulted in disappearance of the phospholipid pre-transition, and broadening and decrease in enthalpy of the main transition. In mixtures containing an excess of HDL, there was complete clearing of turbidity and a single thermal transition with a peak temperature of 25.5 °C (Fig. 2d),

identical to that observed in complexes of delipidated apo-A1 with DML (Fig. 2e). At higher ratios of DML to HDL, there was partial clearing of phospholipid turbidity and thermal transitions with double peaks were observed, the lower temperature component (23.5 °C) corresponding to the gel-liquid crystalline transition of DML multilamellar liposomes (Fig. 2a), and the higher temperature component (25.5 °C) to phospholipid-apoprotein complexes (Fig. 2e).

Phospholipid-apoprotein complexes, prepared from the delipidated apoproteins of HDL, are disks of phospholipid bilayer about 110×55 Å (ref. 15), which typically appear stacked on edge in negatively stained EM preparations¹⁶. Such structures were identified in all incubated mixtures of HDL and DML (arrow, Fig. 3a), and not in control preparations (Fig. 3b). The calorimetric and EM experiments indicate the presence in these mixtures of discoidal DML-apoprotein complexes containing a phospholipid bilayer. In optically clear mixtures (containing no uncomplexed phospholipid), more than 95% of spherical particles observed by EM were between 135 and 210 Å in diameter (for example mean diameter $\pm$ s.e.m. = 145 ± 5.1 Å ($n=150$) in a mixture containing 50% DML/50% HDL apoprotein). Since more than 95% of the spherical particles observed were larger than those present in control HDL preparations (Fig. 3b), an increase in size of the HDL particle appears to accompany formation of phospholipid-apoprotein complexes. This finding was confirmed by recentrifugation at d 1.125 g ml⁻¹

Fig. 2 Differential scanning calorimetry tracings of incubated mixtures of DML and intact HDL: *a*, DML alone; *b*, DML and HDL (DML/HDL apoprotein = 12:1, w/w); *c*, DML and HDL (4:1); *d*, DML and HDL (1:1.2); *e*, DML and apo-A1 (1:1.2). HDL (4–15 mg per ml protein) prepared by centrifugation was added without further concentration to unsonicated turbid suspensions of DML (about 4 mg ml⁻¹). DSC experiments were performed with 75 μ l aliquots of mixtures of HDL and DML incubated under N₂ at 37 °C for 12 h, by which time all changes in turbidity were completed. The tracings were recorded at different sensitivities. Apo-A1 and apo-A1-DML complexes were prepared as described previously¹¹.

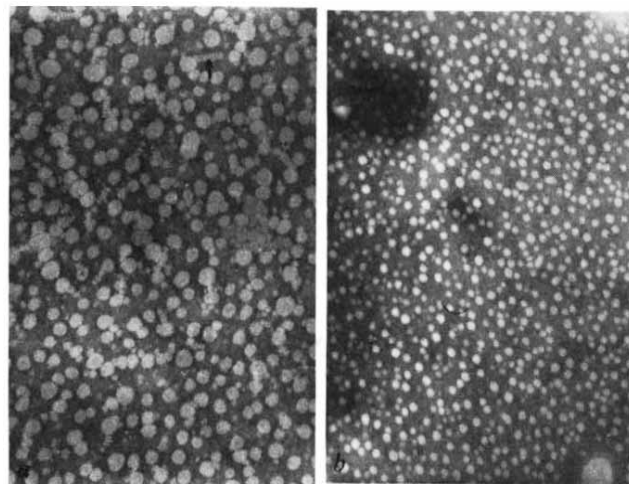
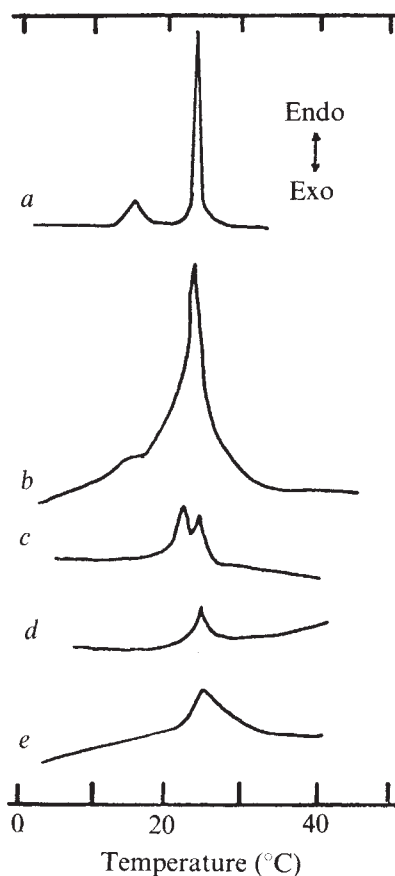


Fig. 3 Electron micrographs, negatively stained with Na phosphotungstate, pH 7.3. *a*, DML and HDL (DML/HDL₃ apo-protein = 2/1). Disks stacked on edge are shown by the arrow. *b*, Control HDL₃ preparation. Magnification of original $\times 33,000$.

of HDL₃ (1.125–1.21 g ml⁻¹) previously incubated with DML. A major portion of the HDL₃ was found to float between 1.063 and 1.125 g ml⁻¹ and compositional analysis showed this was not due to uptake of phospholipid by the HDL₃. The increased size and decreased density of HDL₃ resulting from incubation with DML is consistent with fusion of lipoprotein particles secondary to the removal of apoprotein. Further, the d 1.25 g ml⁻¹ infranatant was found to be enriched in DML and apo-A1, indicating the presence of phospholipid-apo-A1 complexes.

The interaction of the apo-A1 of intact HDL with synthetic phospholipids suggests a possible mechanism for modification of cell membranes. A detergent-like solubilisation of areas of membrane determined by characteristics such as lipid fluidity and cholesterol content could allow an efficient and selective regulation of membrane properties.

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Binding sites related to ouabain-induced stimulation or inhibition of the sodium pump

THE concentration of free cardiac glycosides in the blood of patients treated for heart failure ranges between 1 and 5 nM (refs 1-3). Studies on human heart slices have shown that, within this range of concentrations, digitoxin does not inhibit the sodium pump but does stimulate ^{42}K uptake³. Similar observations have been made with isolated guinea pig atria: low concentrations of ouabain (near 1 nM) stimulate ^{42}K uptake whereas higher concentrations inhibit it⁴. These results are in agreement with recent electrophysiological observations on Purkinje fibres showing that low doses of ouabain produce changes in the K gradient that reflect stimulation of the sodium pump⁵. It has also been observed that the stimulation of the sodium pump by ouabain is associated with a positive inotropic effect⁶. Earlier studies with guinea pig atria¹ and other preparations⁷⁻⁹ showed that the binding of ouabain occurs on non-specific and specific

sites. The specific binding is fitted by a Langmuir curve with an equilibrium constant close to that estimated by measuring the inhibition of the sodium pump. We report here the existence of a binding with a higher affinity associated with the stimulation of the sodium pump.

Experiments were carried out with isolated left guinea pig atria bathed at 30 °C in physiological solution (mM: NaCl 137, KCl (unless otherwise stated) 6, CaCl₂ 1.82, MgCl₂ 0.105, NaHCO₃ 11.9, glucose 5.5) equilibrated with a mixture of 95% O₂ and 5% CO₂. The atria were electrically stimulated at a rate of 3.3 Hz (stimulus of 10 ms, strength at least twice threshold); their resting tension was ± 500 mg.

Figure 1 shows the changes in K and Na contents measured after an incubation of 3 h in the presence of various concentrations of ouabain. At 1 nM and 3 nM ouabain, the Na content was lower and the K content was higher than in control preparations. Since the inulin diffusion space was not modified, these changes reflect a stimulation of the sodium pump. Higher concentrations of ouabain evoked a gain in Na content and a fall in K content, due to the well documented inhibition of the sodium pump^{9,10}.

The uptake of ouabain has been measured in the same experimental conditions. ^3H -ouabain was added together with ^{14}C -inulin in the incubation fluid. This procedure allowed us to correct tissue ouabain uptake for extracellular ouabain content. Cellular ouabain uptake showed both a linear and a saturable component, confirming earlier results obtained with ouabain concentrations ranging from 30 to 10³ nM. This uptake was found to fit the equation¹

$$U = aC_m + \frac{bC_m}{C_m + K_b} \quad (1)$$

where U is the tissue concentration corrected for cardiac

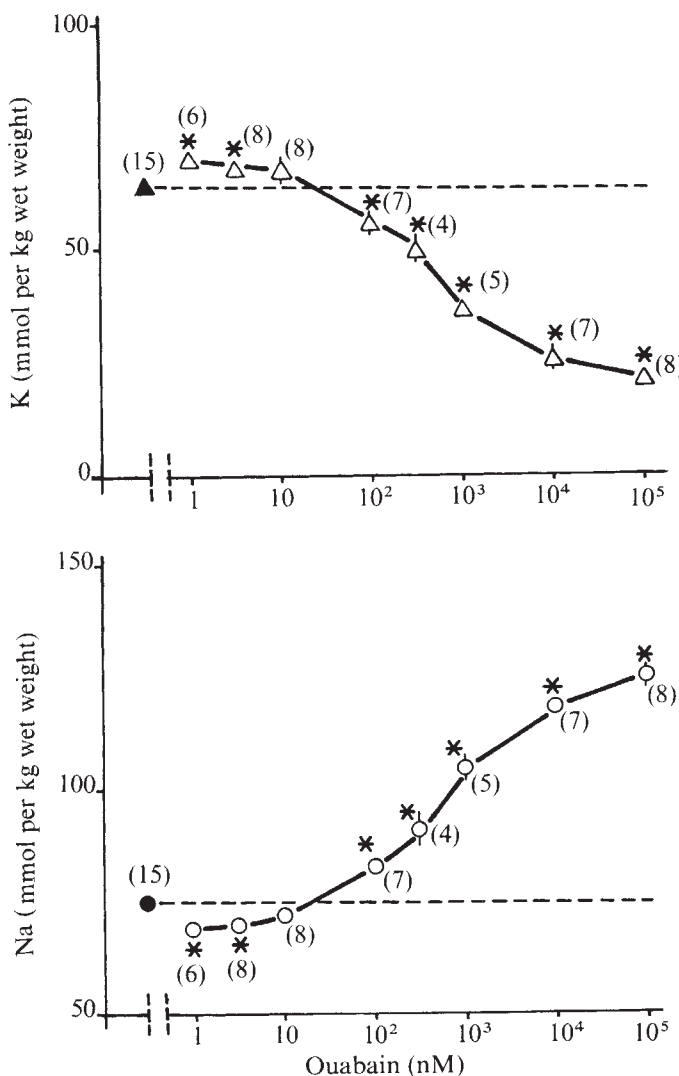


Table 1 Ouabain content of guinea-pig atria corrected for cardiac glycoside in the inulin space

Ouabain medium concentration (nM)	Ouabain cellular content (nmol kg ⁻¹)		P of the difference (observed - calculated)
Observed	Calculated		
a 6 mM KCl			
1	1.1 ± 0.08 (8)	1.3	NS
1.5	2.6 ± 0.2 (8)	1.9	0.001 < P < 0.01
3	5.8 ± 0.5 (8)	3.8	0.001 < P < 0.01
6	10.4 ± 1.0 (8)	7.6	0.02 < P < 0.05
8	10.6 ± 0.67 (7)	10.0	NS
10	12.0 ± 0.67 (8)	12.5	NS
30	37.9 ± 5.1 (4)	36.2	—
10 ²	112 ± 23 (4)	108	—
10 ³	592 ± 48 (4)	587	—
10 ⁴	3,550 ± 126 (4)	3,550	—
b 2.7 mM KCl			
0.5	1.1 ± 0.1 (8)	0.7	0.02 < P < 0.05
0.9	1.5 ± 0.09 (7)	1.2	0.01 < P < 0.02
1	1.7 ± 0.2 (8)	1.4	NS
3	4.7 ± 0.8 (8)	4.1	NS
10	14.1 ± 1.4 (8)	13.1	NS
30	35.5 ± 2.8 (7)	36.3	—
10 ²	96.6 ± 4.0 (7)	96.6	—
10 ³	389.5 ± 13.3 (8)	390	—
10 ⁴	2,279 ± 94.7 (8)	2,279	—

Stimulated left guinea pig atria were incubated for 3 h in the presence of increasing concentrations of ouabain. The ^{14}C -inulin space was estimated for each atrium. The radioactivity was measured as reported before¹¹. Values are presented as means $\pm$ s.e. of mean. The number of determinations is given between brackets. The incubation time of 3 h was sufficient for complete equilibration, except for ouabain 1 nM and 6 mM KCl. For the latter concentration, the equilibrium content reached after 4 h was equal to 2.1 ± 0.09 nmol kg⁻¹ ($n = 8$). The experimental observations are compared with values of U calculated from equation (1) using for the parameters the values estimated for ouabain concentrations between 3 and 10³ nM. The latter were for 6 mM KCl: a (l per kg wet weight) 0.319, b (nmol per kg wet weight) 372, K_b (nM) 389; for 2.7 mM KCl: $a = 0.206$; $b = 219$; $K_b = 193$.

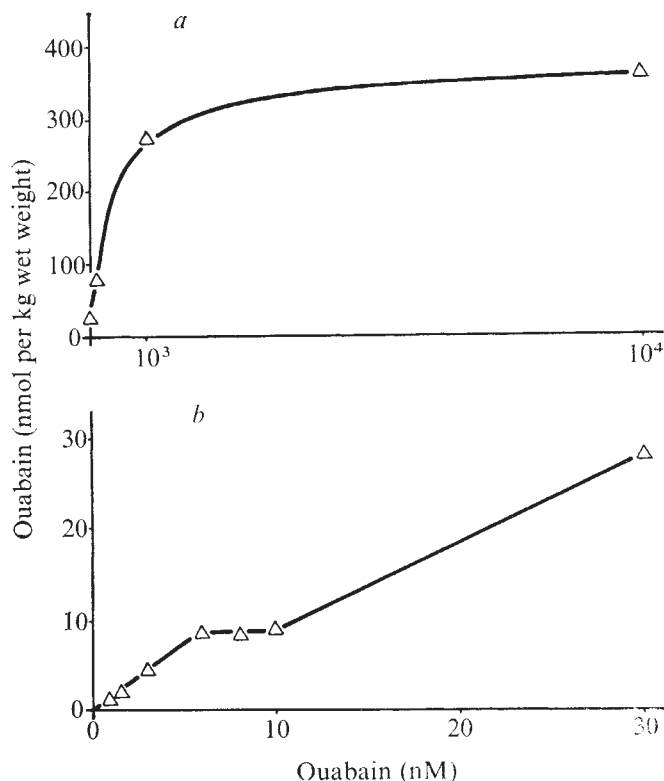


Fig. 2 Specific uptake of ouabain by guinea pig atria. Ordinate: tissue content of ouabain corrected for cardiac glycoside concentration in the inulin space and for the non-specific uptake aC_m . Abscissa: concentration of ouabain in the incubation fluid. Experiments were performed at 30 °C. The ^{14}C -inulin space was estimated for each preparation. The limits of s.e. of mean are shown when they exceed the diameter of the symbol. The number of determinations is given on the graph. Radioactivity was counted as reported before^{4,11}. The lower concentrations in the range covered in *a*, are shown on an expanded scale in *b*.

glycoside content of inulin space, C_m is the cardiac glycoside concentration in the medium, a is the proportionality constant for the linear non-saturable uptake, b is the capacity and K_b is the equilibrium constant for the saturable binding sites. Values of the constants computed as described previously⁴ are reported in Table 1a. The value of a , the non-saturable uptake constant has been estimated in two other experiments. By isotopic dilution, using a large excess of non-radioactive ouabain, constant a was $0.353 \pm 0.0216 \text{ l kg}^{-1}$ ($n = 12$). When ouabain uptake was measured in the presence of a large excess of digitoxin acting as a competitor⁴ the nonspecific uptake of ouabain yielded a value of constant a equal to $0.358 \pm 0.012 \text{ l kg}^{-1}$ ($n = 4$).

The saturable ouabain uptake was obtained by subtracting the non-saturable uptake from the total uptake at ouabain concentrations ranging from 30 nM to 10^4 nM (Fig. 2a). The concentration of ouabain at which half of the saturable binding sites were occupied was equal to 389 nM, a value close to the concentration producing a 50% inhibition of the sodium pump (500 nM).

As shown in Table 1a for ouabain concentrations higher than 10 nM, there was a good agreement between experimental and computed ouabain tissue contents. At ouabain concentrations between 1 nM and 10 nM, ouabain tissue content was higher than that expected from equation (1) using the parameters estimated for ouabain concentrations between 30 nM and 10^3 nM.

Figure 2 illustrates the relation between ouabain concentration and specific ouabain binding. It shows two saturable components: one at concentrations ranging from 30 to 10^4 nM (Fig. 2a) and the other at ouabain concentrations between 1

and 30 nM (Fig. 2b). This indicates the existence of a high affinity and of a low affinity uptake process. The capacity of the high affinity process is only 3% of the capacity of the low affinity one. When the relation between specific binding and ouabain concentration was plotted according to Hill¹⁰, the slope was equal to 2 for the high affinity binding whereas it was 1 for the low affinity binding. This analysis allowed to estimate that the concentration producing a 50% saturation of the high affinity binding was 2.5 nM.

In isolated mammalian cells⁷ and in smooth muscle⁸, extracellular potassium (K_o) competes for both the binding and the inhibitory effect of doses of ouabain higher than 10 nM. Changes in atria excitability, observed when K_o is lower than 2 mM and higher than 8 mM, allowed a limited study of the effect of K_o on high- and low-affinity binding (Table 1). In KCl 2.7 mM, the apparent equilibrium constant of the low-affinity binding was lower than in KCl 6 mM, and the high-affinity component of the binding was observed at lower ouabain concentrations. These observations suggest that the binding of ouabain to both high-affinity and low-affinity sites is to the sodium pump.

The present findings indicate that two populations of sodium pumping sites might coexist. Recent reports have shown that external Na inhibits the sodium pump by an allosteric mechanism^{12,13}. It might be that the high- and low-affinity ouabain binding sites are different states of the same population of receptors. Further experiments are required in order to explore these possibilities.

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Clarification of role of ATP in red-cell morphology and function

RECENTLY there has been a tendency to ascribe many energy-dependent functions of other types of cells to red cells. The widespread belief that red cell form and deformability depend on cellular ATP levels and that membrane internalisation is an energy-dependent phenomenon are cases in point¹⁻⁴. ATP has indeed been shown to control phagocytosis, pinocytosis and locomotion in white cells; the confusion over whether it is pinocytosis (which requires energy) or endovesicle formation (which does not) that occurs in erythrocytes may be due to the observation⁵ of the former phenomenon in erythrocyte precursors (erythroblasts and reticulocytes). To establish that pinocytosis occurs also in the ultimate cell of the series—the erythrocyte—it is necessary to demonstrate that internalisation of the cell membrane is dependent on energy; and thus on temperature, pH, time, and ATP. This is not the case and

results of the investigation described here show that ATP does not directly control either the form and deformability or membrane internalisation of red cells and that phenomena secondary to ATP depletion are responsible for the changes induced.

ATP depletion of red cells was accomplished by incubating washed red cells at 37 °C in either a glucose-free Tris-buffered saline medium or in the same medium containing 1 mM iodoacetate and 0.2% glucose. In both the systems the ATP content of the red cells at various periods of incubation was measured by luciferin-luciferase enzyme assay. The deformability of red cells was determined using the Ektacytometer⁶. Membrane internalisation was produced by treatment with chlorpromazine in the concentration range 0.3 to 0.5 mM, and internalisation of membrane demonstrated by morphological observations using phase contrast, transmission and scanning electron microscopy and by measurement of the deformability and osmotic fragility of cells.

Figure 1 shows the ATP content and form of human red cells as a function of incubating time in two media. In

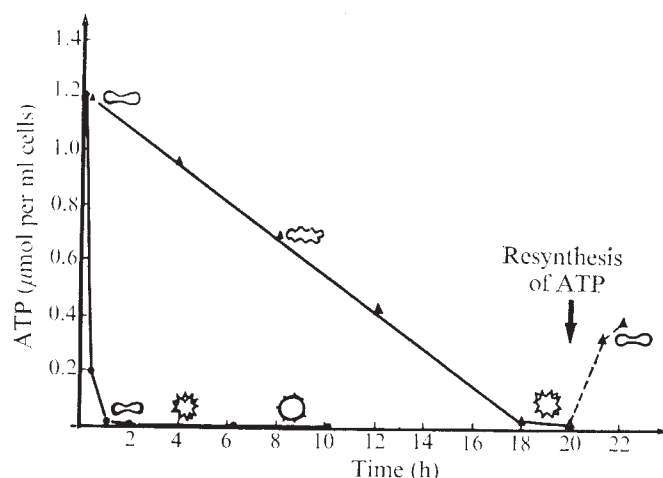


Fig. 1 Changes in ATP content and red-cell morphology with incubation time in different isotonic media: ●, changes obtained by incubation of cells in Tris-buffered saline containing 1 mM iodoacetate. ▲, Changes after incubation in Tris-buffered saline containing no glucose. All the incubations were carried out at an haematocrit of 5%. The pH was maintained at 7.4 and the temperature at 37 °C during the whole course of incubation.

the glucose-free medium the discocyte-echinocyte transformation occurs by a progressive increase of echinocytes which are formed in the presence of discocytes, thus demonstrating heterogeneity of the morphology of the population before complete ATP depletion.

When erythrocytes were incubated in a medium with

iodoacetate it was possible to maintain discocytic red cells with zero ATP. By prolonging incubation time to 3 or 4 h, a progressive and complete discocyte-echinocyte transformation took place in the presence of iodoacetate. This shows that shape changes will ultimately, though not initially, be caused by effects secondary to ATP depletion that is; by alteration of the ionic equilibrium, loss of cations, and modification of the integrity of the membrane.

For the same cellular concentrations of ATP we can obtain red cells in various morphological states, suggesting strongly that the ATP concentration *per se* does not control cell shape and that ATP is only necessary for maintaining the integrity of other systems that are responsible for the discocytic form. Weed⁷ has postulated that ATP-Ca²⁺ ratio is important in controlling form and deformability; and although we have not measured Ca²⁺ levels in the cells during these incubations, we have obtained identical results with incubating media containing chelating agents EDTA and EGTA which were used for the purpose of ruling out the possibility of external Ca²⁺ penetrating the cells during or after ATP depletion. Deformability data shown in Table 1 demonstrate that normal discocytes and discocytes with zero ATP have identical deformabilities. In both of the incubating media reduction in deformability could only be detected after the spherocyte stage has been reached. Measurements of red cells treated with lyssolecithin permitted us to evaluate deformability changes in echinocytes with normal ATP levels. Here again, decrease in deformability could only be detected after the spherocyte stage had been reached.

Deformability of red cells measured at 4 °C and 37 °C was almost identical.

A dilute suspension (1%) of washed human red cells (discocytes) treated with 0.3 mM chlorpromazine underwent membrane internalisation and were transformed into spherocytes. These spherocytes with endovesicles (Fig. 2) were undeformable (Table 1) and had increased sensitivity to osmotic stress (50% haemolysis at 225 mOsm compared to 50% haemolysis at 140 mOsm for discocytes). When we treated discocytes having no ATP with 0.3 mM chlorpromazine we obtained exactly the same results. These spherocytes also had endovesicles (Fig. 2), reduced deformability and identical increased sensitivity to osmotic stress. Thus the absence of a role for ATP in membrane internalisation in red cells was clearly demonstrated.

The results presented here indicate that in our experimental conditions with intact red cells a direct role of ATP in red-cell membrane function could not be demonstrated. To demonstrate that membrane internalisation could be an energy-dependent phenomenon one should avoid inducing ATP depletion and at the same time a change of red cell shape. According to Deuticke a fixed concentration of chlorpromazine forming vacuoles in a discocyte would not be active on an echinocyte; that is, endocytosis would not

Table 1 Correlation between red-cell shape, ATP content and ability to deform in a free shear field

Red-cell shape	ATP content (μmol per ml RBC)	Average red-cell dimensions*	
		No stress (μm)	At a shear stress of 750 dyne cm ⁻² (μm)
Discocytes	1.2	8.0	16.5
	0.0	8.0	16.5
Echinocytes III	1.2 LPC	5.5	16.0
	0.0	5.5	16.0
Sphero-echinocytes	1.2 LPC	5.5	8.0
	0.0	5.5	8.0
Sphero-stomatocytes with endovesicles	1.2	5.5	6.5
	0.0	5.5	6.5

Red-cells were suspended in a viscous dextran solution (0.14 Poise) and subjected to a shear stress of 750 dyne cm⁻². LPC = lysophosphatidylcholine-treated red cells.

*The average red-cell dimensions represent either the diameter of the disk or sphere when no stress is applied, or—when cells are deformed—the size of the larger axis of the ellipse obtained under stress.

occur in the latter cell. In fact we can show that echinocytes with normal or reduced ATP levels can be induced to form endovesicles at higher concentrations of chlorpromazine.

Another argument against energy requirement for membrane internalisation is that identical endovesicle formation

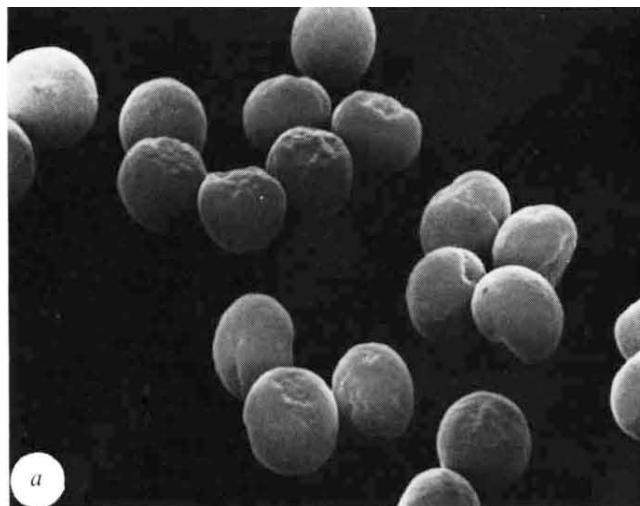


Fig. 2 *a*, Spherostomatocytes obtained by treating normal human discocytes with 0.3 mM chlorpromazine as seen after fixation in scanning electron microscopy. *b*, Endovesicles in spherostomatocytes after treatment with chlorpromazine of red cells containing normal ATP as seen in transmission electron microscopy. *c*, Endovesicles in spherostomatocytes containing no ATP. Red cells were incubated at 37 °C with 1 mM iodoacetate, then treated with chlorpromazine and observed using electron microscopy.

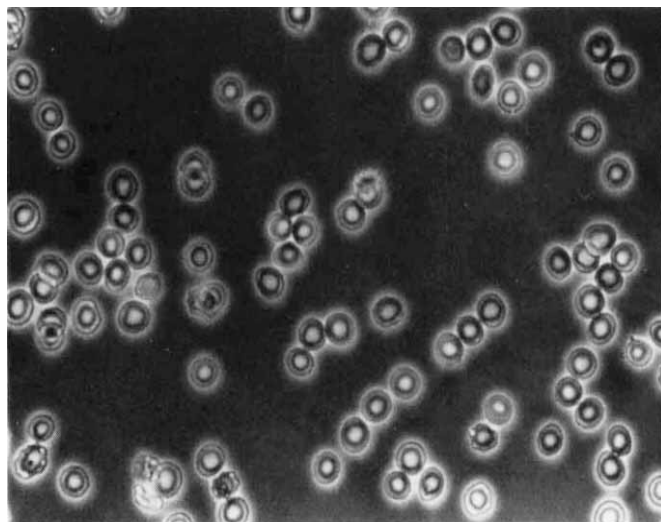


Fig. 3 Red cells incubated 1 h at 37 °C in a Tris-buffered medium containing 1 mM iodoacetate; ATP was $\leq 0.05 \mu\text{mol per ml}$ red cells. Cells were fixed in 1% glutaraldehyde and observed with a phase contrast microscope (magnification $\times 290$). 95% of the cells are discocytes, 5% are echinocytes 1 to 3.

was obtained at 4 °C and 37 °C. Neither was pH dependence found, in particular for low values compatible with complete solubility of the drug. A false pH dependence should result from precipitation of the drug at figures above 7.4.

A last point is the absence of time dependence since vacuoles are abundantly formed very rapidly (within seconds) as is shown by using high concentration of the chemical agents and rapid fixation of the red cells. During the same experiment no measurable ATP depletion could be detected.

Our results are difficult to compare with those using ghosts, as it can be considered that haemoglobin-free red cells represent a very different model.

In contrast, white cell deformability, phagocytic capacity and locomotion are all strong functions of temperature and are inhibited by depletion of cellular ATP levels. Our results, which conclusively show that red-cell form, deformability and membrane internalisation are not energy-dependent functions, taken together with those of others for white cells, strongly suggest that these two cells have different membrane systems for controlling these functions.

We think our data are most easily interpreted by a physicochemical hypothesis as proposed by Singer and Sheetz⁸. According to them, amphiphilic molecules are asymmetrically distributed in the membrane, that is preferentially in the interior part of it for cationic compounds, thus leading to shape changes and vacuole formation.

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Do androgens increase Leydig cell sensitivity to luteinising hormone?

TARGET tissue responsiveness to hormones is not a static phenomenon, but is often influenced by the endocrine situation. For example, the sensitivity of the Leydig cell in the rat to luteinising hormone (LH) stimulation is not constant but gradually increases during prepubertal development¹. Moreover, during treatment of hypogonadism in the human male, human chorionic gonadotrophin (hCG) therapy is often increasingly efficacious in stimulating testosterone secretion after repeated injections—a probable manifestation of induced target cell sensitivity. It is possible that androgen receptors in the Leydig cell^{2,3} are involved in the regulation of these changes in sensitivity. They are specific for testosterone and dihydrotestosterone^{4,5} and are distinct from the oestrogen receptors in the same cell^{3,6}. We have investigated a possible role for these androgen receptors using the testicular feminised male (Tfm) rat. This rat has a male genotype and abdominal or inguinal testes, but a typical female phenotype because of a genetically determined lack of androgen receptors and a generalised target organ insensitivity to androgen⁷. We report here that the Leydig cells of the Tfm rat testis have much smaller number of LH/hCG receptors than normal, and this is associated with a low sensitivity to hCG.

To determine whether the responsiveness of Leydig cells of Tfm rats to hCG differed from that of normal males, we incubated Tfm and normal testes with the same dose of hCG. Figure 1 shows the response in terms of testosterone released into the medium. The Tfm testes exhibited a markedly impaired response, with treated testes releasing the same quantities of androgen as did control, unstimulated testes.

What defines the level at which a tissue will respond to a hormone stimulus? Most workers agree that the number of hormone receptors is one of the primary factors controlling the degree of responsiveness of a target tissue to a hormone. Therefore we investigated whether the Tfm testis had a normal content of LH receptors. Figure 2 shows Scatchard plots and saturation curves of LH binding to membrane receptors isolated from the testes of Tfm rats and their normal litter mates (NL). Although LH receptors in

Fig. 1 The effect of hCG on testosterone secretion from the testes of Tfm and normal litter mates (NL). Decapsulated testes from Tfm and NL rats ($n = 5$) were incubated with or without 3 ng of hCG in 2 ml of Eagle's tissue culture medium. One testis from each animal was incubated in the presence of hCG and the contralateral testis served as control (without hCG). Incubation was performed for 4 h at 34 °C in a Dubnoff shaker, with a gas phase of 95% O₂ and 5% CO₂. Aliquots (25 μ l) were taken from media after various times and testosterone was determined by radioimmunoassay. Vertical bars = standard deviation. ■, NL testes with hCG; □, NL testes without hCG; ○, Tfm testes with hCG; ●, Tfm testes without hCG.

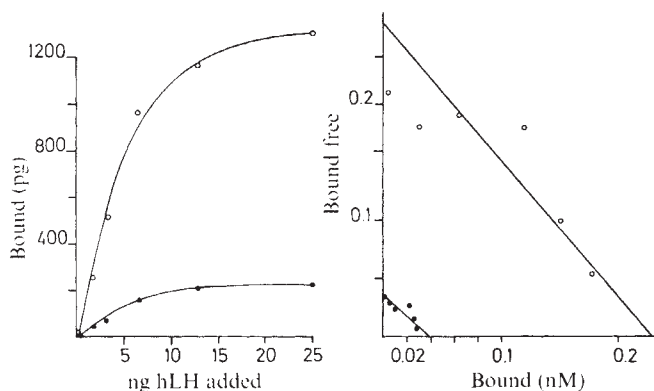
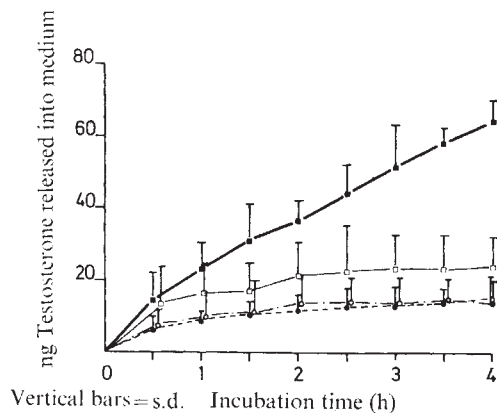


Fig. 2 Binding of human luteinising hormone (hLH) (15,000 IU per mg) to membrane receptors from the testes of Tfm (●) and NL (○) rats. Testes were homogenised in 10 volumes of phosphate-buffered saline (PBS), pH 7.4, and centrifuged at 27,000g for 30 min. The pellets were washed in the same buffer, recentrifuged and finally resuspended in 3 volumes of PBS containing 0.1% bovine serum albumin (BSA) and 0.1% Neomycin. Fifty μ l (300 μ g of protein) of membranes were equilibrated at 22 °C for 20 h with 10,000 c.p.m. of ¹²⁵I-hLH and various amounts of non-labelled hLH (0–20 ng) in a total volume of 200 μ l. The pellets were centrifuged at 5,000g for 30 min, washed three times in the same buffer and the final pellets counted in a Packard Auto Gamma with 50% efficiency. Five μ g of ovine LH (NIH-LH-S19) was used to assess nonspecific binding (6% of total binding). The data were plotted according to Scatchard (bound/free versus bound)¹⁵ and the x-axis intercept gives the binding capacity.

normal and Tfm testes had similar binding affinities, there were many fewer in the Tfm testes, which had approximately 20% of the number found in the normal situation when expressed in fmol per mg of protein and 5–10% when calculated per testis. As there seem to be more Leydig cells in the Tfm rat testis than in normal testicular tissue⁸, these results suggest a considerable difference in the number of LH receptors per cell. The lack of response of the Tfm testis to hCG is probably due to the reduction in the number of LH receptors on the Leydig cell membranes. The fact that the Tfm syndrome is characterised by a reduced ability of androgens to exert a physiological and biochemical effect indicates that, in the normal situation, androgens are necessary for the normal induction of LH receptors in the Leydig cell. It is possible that this effect is mediated through the androgen receptor since the Tfm Leydig cells, contrary to the normal situation, have no androgen receptors⁵.

Recent studies in our laboratory suggest that this induction takes place during a critical phase of prepubertal development. Treatment of immature male rats with a pure antiandrogen (cyproterone) results in a significant reduction in intratesticular and plasma testosterone without concomitant reduction in plasma gonadotrophin⁹. Similar treatment in the adult male stimulates testosterone secretion due to a central antiandrogen influence¹⁰. The fact that in the Tfm mouse the Leydig cells are arrested in their cytological development⁷ strengthens the evidence for a short-loop positive influence of androgens on the Leydig cells during postnatal differentiation. The human Tfm syndrome, which is similar to that found in rats and mice, is associated with castration levels of LH¹¹ due to the impaired androgen feedback on the pituitary and hypothalamus. Plasma testosterone levels, however, are in the normal or subnormal male range^{12,13}, suggesting that Leydig cells in the human Tfm syndrome also show a reduced sensitivity to LH.

Study of the hormonal induction of peptide hormone receptors is the first step to an understanding of the mechanisms underlying fluctuations in tissue responsiveness. Androgens seem not only to induce LH receptors in the Leydig cell but also to stimulate the concentration of prolactin receptors in the rat prostate gland¹³, indicating that the receptor-inducing action of the same steroid may be diverse and very complex.

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Role of prostaglandin endoperoxide PGG₂ in inflammatory processes

SINCE the discovery that non-steroidal anti-inflammatory agents (NSAI) are potent inhibitors of prostaglandin synthetase¹⁻³, there has been controversy about the role of primary prostaglandins (PGEs+PGFs=PGs) in inflammatory diseases. A parallel has been demonstrated between the ability of drugs such as indomethacin and aspirin to inhibit cyclo-oxygenase, the initial step leading to the formation of PGs, and anti-inflammatory activity⁴⁻⁶. The failure of administered PGs to fully mimic inflammation processes, however, is not consistent with this concept^{7,8}, and further studies have shown that other products derived from the reaction of cyclo-oxygenase with arachidonic acid (PGG₂, PGH₂ and thromboxane A₂) although quite labile, have biological activities exceeding the potency of the PGs themselves^{9,10}. Since these ephemeral mediators are also subject to regulation by NSAI they too must be considered as potential causal agents of inflammation. We have investigated this possibility by examining the action of several NSAI on the total pattern of arachidonic acid oxygenation products. The results indicate that the endoperoxide PGG₂ has a pivotal role in acute inflammation.

During a search for potential non-steroidal anti-inflammatory drugs, one was found to possess a high degree of biological activity, yet it was inactive as an inhibitor in the routine PG synthetase assay⁴. In rat foot oedema, this compound, 2-aminomethyl-4-*t*-butyl-6-iodophenol (MK-447), exhibited good anti-inflammatory activity. Figure 1 shows the dose-response line for oral dosage compared with those for indomethacin and phenylbutazone. The ED₅₀ values for indomethacin, MK-447 and phenylbutazone were 2.73, 17.3 and 27.7 mg kg⁻¹ respectively. At the level of 50% inhibition of the foot oedema therefore MK-447 was approximately

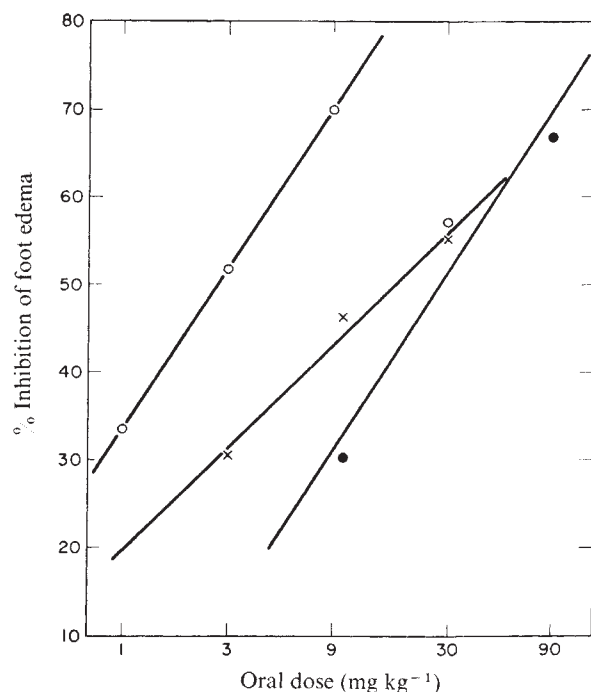


Fig. 1 Comparison of the anti-inflammatory activity of MK-447 (×) with the PG synthetase inhibitors indomethacin (○) and phenylbutazone (●) on carrageenan-induced oedema in the rat foot. For indomethacin $y = 38.04 \log x + 33.43$ ($n = 780$ rats); MK-447, $y = 24.5 \log x + 19.6$ ($n = 96$ rats); phenylbutazone, $y = 38.54 \log x - 5.61$ ($n = 306$ rats). Male Sprague-Dawley rats (150–170 g body weight) were used in groups of 6 each. Compounds to be tested were given by stomach tube in a water solution of 0.5% Methocel used as a suspending agent; 3 ml were given per rat. One hour later, 0.1 ml of 1% carrageenan suspension (Viscarin, Marine Colloids) was injected subcutaneously under the plantar surface of the right hind foot. The volume of the foot was determined immediately by immersion of the foot into mercury up to a previously marked ink spot; the volume of mercury displaced was recorded automatically. The mark was placed on the skin over the lateral malleolus. Three hours later, the volume was measured again; the difference between the two volumes was the swelling. Drug effects were calculated as percentage inhibition of the swelling, taking the swelling of the control group as 100%.

one-sixth as potent as indomethacin, and 1.6 times as potent as phenylbutazone. A similar high degree of activity in suppressing the swelling of the mouse's ear induced by croton oil¹¹ was also noted. Prostaglandin synthetase inhibitors are efficacious in both assays. It is interesting that the dose-response slopes for MK-447 in both the foot oedema and mouse ear assay, 24.5 and 47.0 respectively, differ from those of the two PG synthetase inhibitors, indomethacin (38.0 and 65.0) and phenylbutazone (38.5 and 65.0). This difference suggested a mode of action for MK-447 not involving inhibition of PG synthetase.

As noted, MK-447 did not inhibit the synthesis of PGs from arachidonic acid when a ram seminal vesicle preparation was used, nor did it inhibit the reaction when oxygen uptake was monitored. In fact, in the absence of cofactors, MK-447 stimulated overall prostaglandin synthesis in a microsomal preparation derived from ram seminal vesicles (Table 1). A similar ability to stimulate the synthesis of PGs was observed in incubated intact mouse ovaries and rat kidney slices (data not shown). These observations indicating that both inhibitors and stimulators of the formation of prostaglandins can suppress inflammatory responses can be interpreted to mean that an intermediate in their formation, apart from the prostaglandins, has a causal role in acute inflammation. The formation of such an intermediate would be expected to be blocked by PG synthetase inhibitors which act at the initial step in the sequence. Levels of such an intermediate would be depressed by the MK-447-facilitated conversion to PGs. In the events leading to the formation of

Table 1 Effects of non-steroidal anti-inflammatory agents on endoperoxides and PGs derived from enzymatic oxygenation of arachidonic acid

Product	Incubation time (min)	No additions	MK-447 (100 μ M)	nmol per mg protein Indomethacin (10 μ M)	Phenylbutazone (100 μ M)	Aspirin (100 μ M)
AA	0.5	23	10	43	24	44
PGG ₂	0.5	16	6	1	0	0
PGH ₂	0.5	4	27	0	20	0
AA	5	7	7	44	22	44
PGG ₂	5	13	0	0	4	0
PGH ₂	5	5	18	0	11	0
PGs	5	8	15	0	7	0

Values in each category represent a minimum of five experiments. Total radioactivity recovered was greater than 75% in each case and reproducibility of the values for each individual experiment was $\pm 6\%$. Microsomes were prepared from ram seminal vesicles as described before¹⁴. The compounds were preincubated with 500–600 μ g of microsomal protein in 0.2 ml of 0.1 M potassium phosphate buffer, pH 7.5, for 5 min at 25 °C. The reactions were initiated by the addition of labelled arachidonic acid (22 nmol containing 0.25 μ Ci of ¹⁴C-1-arachidonic acid) and incubated for the times indicated. They were terminated by adding 0.05 ml of 0.3 M citric acid, and the products and remaining arachidonic acid were extracted into ether precooled to –70 °C. The entire procedure was carried out in the cold. Ether extracts were chromatographed at –20 °C on Whatman SG-81 silica-impregnated paper using ether–hexane–acetic acid (85:15:0.1) as described²⁴. Incubation for 0.5 min resulted in the formation of only trace amounts of PGE₂ and PGF_{2 α} .

PGs from arachidonic acid (AA), that is AA $\rightarrow$ PGG₂ $\rightarrow$ PGH₂ $\rightarrow$ PGs, either PGG₂ or PGH₂ would qualify as such an inflammatory agent. The overall sequence of these reactions is depicted in Fig. 2.

As Table 1 shows, approximately half the arachidonic acid is utilised by the microsomal enzyme leading to the formation chiefly of PGG₂ during a 0.5-min incubation. The formation of both endoperoxides is almost completely suppressed by indomethacin. This finding is consistent with an inhibitory effect on the cyclo-oxygenase step¹², the initial reaction in the oxidation of arachidonic acid by PG synthetase. Phenylbutazone inhibits the cyclo-oxygenase reaction less effectively, but significantly alters the ratio of endoperoxides in favour of PGH₂. Aspirin, at relatively high levels, blocks the formation of PG intermediates, a result consistent with an action to acetylate the cyclo-oxygenase¹³. Finally, MK-447 facilitates the enzymatic oxidation of AA to the endoperoxide PGH₂ in 0.5 min and

ultimately to prostaglandins as reflected by the value obtained with the longer incubation.

The data presented here show that the quantitative effects of the NSA1 studied differ markedly. But these compounds consistently and considerably depress levels of PGG₂, in spite of various effects on the other parameters even including a stimulation of the formation of PGs by MK-447.

Studies of several analogues of MK-447 (data not shown) revealed an obligatory requirement for the free phenolic group for both enzymatic and biological activity; the methyl ether was completely inactive in both parameters. Since phenol itself has been shown to stimulate both the uptake of oxygen by the cyclo-oxygenase and the overall synthesis of prostaglandins¹², a mechanism of action common to both MK-447 and phenol is suggested.

The capacity of phenol to stimulate the synthesis of prostaglandins has been reported to be linked to its ability to facilitate the conversion of the endoperoxide PGG₂ to

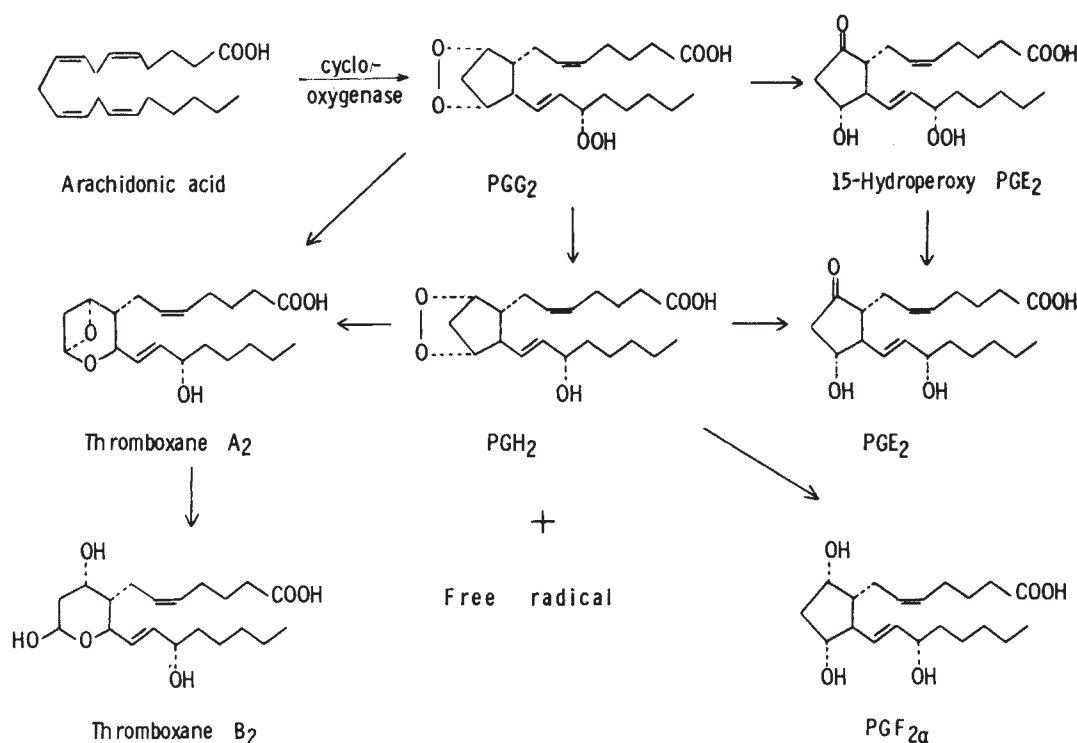
**Fig. 2** Enzymatic conversion of arachidonic acid to potential mediators of inflammation.

Table 2 Anti-inflammatory activity of phenol in the mouse ear assay

Phenol (mg)	PMA (mg)	Phenol application relative to PMA	Average change (mg)*	% Inhibition	P
0.25	0	—	0.15±0.07	—	—
0.5	0	—	0.13±0.38	—	—
1.0	0	—	1.02±0.39	—	—
0	0.05	—	14.8±1.3	—	—
0.33	0.05	15 min pre.	11.0±1.0	25.7	<0.05
0.33	0.05	with PMA	10.4±0.71	29.7	<0.01
0.33	0.05	1 h post	10.8±1.73	27.0	<0.05
0.33	0.05	2 h post	12.6±1.3	14.9	NS
1.0	0.05	15 min pre.	6.9±0.68	53.4	<0.01
1.0	0.05	with PMA	8.4±1.54	43.2	<0.01
1.0	0.05	1 h post	10.2±2.4	31.1	<0.10
1.0	0.05	2 h post	12.7±2.0	14.2	NS

The mouse ear oedema assay¹¹ was used for these studies, but modified in the use of phorbol 12-myristate-13-acetate (PMA) in place of croton oil as the challenging agent. Groups of 6 mice were used for measurements of the action of phenol and PMA alone (each in 0.1 ml of acetone) and in combination. At times indicated after this challenge a second group received the acetone solution of phenol. Four hours later the mice were killed by cervical dislocation, 8-mm circles of ear were punched out, maintained in standard conditions of humidity and weighed.

*Values represent the increase in weight of the treated compared with untreated ear.

PGH₂ (ref. 14). The corresponding methyl ether is inactive. This effect on the endoperoxide interconversion is a property common to both phenol and methional, a well recognised radical scavenger. Electron paramagnetic resonance studies showed that phenol acts as a free radical scavenger in this reaction, causing stimulation of overall PG synthesis by sequestering the destructive oxygen-centred radical released in the conversion of PGG₂ to PGH₂ (ref. 14). Removal of this radical by phenol not only facilitates the conversion of PGG₂ to PGH₂ but also increases the net turnover of arachidonic acid by minimising oxidative inactivation of cyclo-oxygenase by this same destructive free radical.

Although phenol is inherently quite corrosive to tissues it has been used topically as a pain killer at low doses¹⁵. We do not know, however, of any report that phenol represses the oedema involved with inflammatory processes. Because of the property common to MK-447 and phenol in stimulating the conversion of PGG₂ to PGH₂ it was interesting to investigate whether phenol itself, like MK-447, had anti-inflammatory activity as measured in one of the *in vivo* test models of inflammation. We used the mouse ear oedema assay¹¹, but modified it by using phorbol myristate acetate (PMA), a component of croton oil, as the challenging agent. At levels up to 1 mg per site, applied topically, phenol had no significant effect on ear weight by itself when measured 4 h after application (Table 2). At this level a 53.4% reduction in tissue weight occurred when phenol was applied 15 min before the PMA challenge. Significant inhibition was also noted when phenol was applied 1 h after treatment with PMA and read at 4 h. Thus the ability of phenol to mimic the anti-inflammatory properties of MK-447 and their common actions to stimulate the conversion of PGG₂ to PGH₂ and overall PG synthesis are supportive of the concept that the mechanism of action of MK-447 relates to its ability to scavenge free radicals.

The most salient feature of these studies on the oxygenation of arachidonic acid is that levels of the endoperoxide intermediate, PGG₂, alone are depressed by all drugs examined. These data imply that PGG₂ has a pivotal role in acute inflammation. A particularly cogent finding is that one of these drugs, MK-447, can not only cause a depression of PGG₂ in an *in vitro* enzyme system, but also elicit an increased synthesis of PGEs by both microsomal enzyme and by incubated tissues. Thus the demonstrated anti-inflammatory activity of this compound in animal models of acute inflammation, coupled with its ability to stimulate the synthesis of PGs, is not compatible with the concept that primary prostaglandins (PGEs and PGFs) play a key role in inducing the symptoms of inflammation.

Since vasodilation, one component of acute inflammation, is well duplicated by administration of PGE₁ alone^{16,17} it is

reasonable to suggest that this aspect of the disease relates to the formation of PGEs. But the failure of administered PGEs to fully mimic the oedema^{18,19} and pain²⁰ of inflammation points to PGG₂ as a prime factor in these two components of acute inflammation in view of the data presented here. In focusing on the endoperoxide it is necessary to consider that PGG₂ itself may not be the actual triggering agent in acute inflammation, but merely the precursor of such a substance. Such potential causal agents are shown schematically in Fig. 2. Thromboxane A₂, recently identified as the potent rabbit-aorta-contracting substance, is formed from PGG₂, and its derivation from this endoperoxide may be the preferred pathway¹⁰. Alternatively, the free radical formed in the metabolism of PGG₂ and tentatively identified as a hydroxy radical¹⁴ may be the inflammatory component liberated in the enzymatic oxidation of arachidonic acid. Such hydroxy radicals have been reported to be very destructive to biological systems and their actions have been linked to inflammatory processes^{21,22}. Evidence that peroxy acids are extremely effective pain-inducers²⁰, and that increased lipid peroxidation accompanies release of lysosomal enzymes²³ adds further credence to the concept that the hydroxy radical may be the relevant oxygenation product of AA in inflammatory diseases. Finally, it is necessary to consider hydroperoxy PGE₂ which also derives from PGG₂. Like PGG₂, 15-hydroxyperoxy PGE₂ is potentially able to liberate an oxygen-centred free radical. In any case we conclude from these studies that the primary PGs as represented by PGE₂ and PGF₂α are not the important inflammation-inducing agents obtained from arachidonic acid. Rather the endoperoxide, PGG₂, itself or a non-prostaglandin product(s) derived from it seems to be the major inflammatory agent formed during the enzymatic oxidation of arachidonic acid and blocked by the action of PG synthetase inhibitors, resulting in improvement of the symptoms of inflammation.

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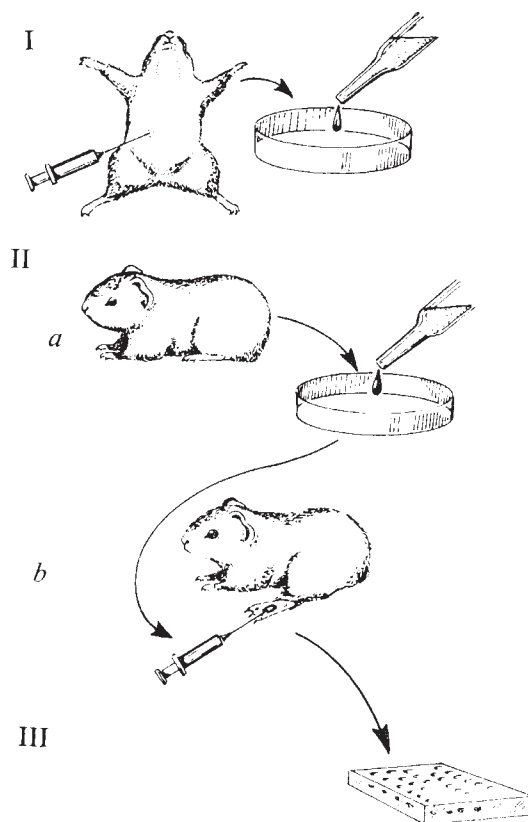


Fig. 1 Experimental format for study of the regulation of auto-sensitisation to BP. I, BP-pulsed macrophages. Peritoneal exudate macrophages were prepared from unprimed Strain 13 guinea pigs, 4 d after intraperitoneal injection of 15 ml sterile Drakeol, by lavaging the peritoneum with sterile phosphate-buffered saline (PBS) plus heparin, 5 units ml⁻¹. All procedures were then performed in RPMI + 25 mM HEPES buffer without serum at 37 °C; 20×10^6 macrophages were allowed to adhere to 100-mm Petri dishes for 2 h, then washed to remove non-adherent cells. The macrophages were then pulsed for one hour with 100 µg ml⁻¹ of either BP or G.T. Antigen not associated with macrophages was removed by changing the medium three times. II, Sensitisation of initiator lymphocytes. a, 100×10^6 unprimed spleen cells were added to the macrophage monolayers. Some groups received soluble BP at a concentration of 100 µg ml⁻¹ during lymphocyte-macrophage interaction. After 16 h of incubation of the lymphocytes on the macrophage monolayers, non-adherent cells were removed, then replated for 1 h to re-adhere detached macrophages. No more than 2.5% of the recovered cells seemed to be macrophages and more than 95% were small lymphocytes. The initiator lymphocytes were irradiated with 1,000 R to prevent their replication but not their ability to recruit *in vivo*⁶. b, Lymphocytes were then injected into the footpads of syngeneic animals at a concentration of 10×10^6 cells in 0.1 ml PBS. Some animals received injections of 10^6 antigen-pulsed macrophages in 0.1 ml PBS into the footpads. The macrophages were obtained from the plates by scraping with a rubber policeman. Each group contained three or four guinea pigs. III, Assay of proliferative response of recruited lymphocytes. After 13 d the draining popliteal nodes were excised and lymph node cell suspensions of each group were pooled. After 1 h of adherence on plastic Petri dishes to remove debris and dead cells, 5×10^5 cells were placed in culture on Greiner microtitre plates. Medium for the assay phase consisted of RPMI + 25 mM HEPES + 1.25% foetal calf serum, 5×10^4 unprimed, unstimulated macrophages were irradiated with 2,000 R and added to each culture. Cultures were incubated with 15 µg ml⁻¹ of various test antigens for 72 h, then 1 µCi of ³H-thymidine was added for 16 h. Cultures were then collected on a MASH 2 cell harvester and counted in scintillation fluid in a Packard Beta counter. Results are reported as the mean and standard error of the four or five culture wells which comprised each group.

Regulation of autosensitisation to encephalitogenic myelin basic protein by macrophage-associated and soluble antigen

AUTOIMMUNE diseases are characterised by an attack of an individual's lymphocytes against his own body's components, self-antigens. The immunogenicity of self-antigens can be defined operationally by their capacity to trigger lymphocytes bearing specific receptors. We have designed experiments to learn how a defined self-antigen can function as an immunogen *in vitro* and how the form of its presentation to self-recognising lymphocytes may abrogate this immunogenicity. We have found that myelin basic protein (BP) behaves as a self-immunogen when presented to lymphocytes by way of syngeneic macrophages. Moreover, recognition of this self-immunogen can be inhibited by the presence of the same self-antigen in soluble form during the primary lymphocyte-macrophage interaction.

BP is a self-antigen whose amino acid sequence and encephalitogenic determinants have been characterised¹. Injection of BP in a suitable adjuvant produces in susceptible animals an autoimmune disease called experimental allergic encephalomyelitis (EAE) (ref. 2). The disease is manifested by histological evidence of lymphocytic infiltration of the central nervous system and is expressed clinically by paralysis. For induction of EAE, BP is usually injected in complete Freund's adjuvant; when the same self-antigen is administered in a soluble form without an adjuvant, it is not encephalitogenic. Moreover, under certain circumstances, treatment with soluble BP or its chemical analogues may suppress EAE (refs 3-5). Thus, the development of EAE seems to require suitable presentation of BP to the immune system.

To analyse the immunogenicity of BP, we focused on the initial phase of lymphocyte recognition of BP and separated this process from the subsequent differentiation of effector lymphocytes. We extended a previous observation that recruitment of immunospecific recipient lymphocytes can serve as a means of detecting whether recognition of self-antigens by initiator lymphocytes has occurred *in vitro*⁶. Thus, the recognition of BP was detected by testing the ability of the initiator lymphocytes to specifically recruit lymphocytes in highly inbred strain 13 guinea pigs. A three-phase system was developed (Fig. 1). In stage I monolayers of macrophages were prepared and pulsed with BP antigen. In stage II, the *in vitro* sensitisation phase, initiator lymphocytes were exposed to the BP-pulsed macrophages in the presence or absence of soluble BP. The initiator

lymphocytes were then collected, irradiated to prevent their proliferation, and injected into syngeneic recipient guinea pigs. In stage III, the assay phase, the DNA proliferative response of recruited lymphocytes to BP served to indicate whether autosensitisation of the initiator lymphocytes had occurred.

The results in Fig. 2 demonstrate that BP was recognised, and triggered syngeneic spleen lymphocytes when presented on macrophages. Initiator lymphocytes were exposed to BP-pulsed macrophages and the lymphocytes were injected into recipient animals. Thirteen days later, recruited lymphocytes from the draining popliteal nodes of the recipient animals reacted to BP with a stimulation index (SI) of 4.4 (Fig. 2). The recruited lymphocytes did not respond to lysozyme, a protein of similar molecular weight and charge. Furthermore, lymphocytes incubated on macrophages not pulsed with antigen failed to recruit lymphocytes responsive to either BP or lysozyme, excluding a potential nonspecific stimulation resulting from the lymphocyte-macrophage interaction.

A control was designed to test whether passive transfer of BP, rather than *in vitro* sensitisation of the initiator lymphocytes could account for recruitment of recipient lymphocytes. Guinea pigs were injected with 500 μg of BP, a dose equivalent to the entire amount present in the *in vitro* culture. The recipient animals showed no evidence of sensitisation to BP (Fig. 2), suggesting that mere passive transfer of antigen could not have triggered active sensitisation to BP *in vivo*. Another control was done to test the possibility that antigen-bearing macrophages that had contaminated the injected lymphocytes acted as the initiators of recruitment. Recipients were injected with 1×10^6 macrophages that had been incubated with BP. This number of macrophages was at least 4 times greater than the number of macrophages actually observed to contaminate the injected lymphocytes. The response to this dose of macrophages was also insignificant (Fig. 2). In other experiments (unpublished) we found that injection of 5×10^6 or more BP-pulsed macrophages was needed to sensitise guinea pigs against BP *in vivo*. Thus, autosensitised initiator lymphocytes and not passively transferred antigen nor antigen associated with macrophages induced the recruitment of recipient lymphocytes against BP in these experiments.

Fig. 2 Immunogenicity of BP on syngeneic macrophages. Incorporation of ^3H -thymidine was measured in recruited lymphocytes from the draining popliteal lymph nodes of recipients, 13 d after injection. Recipients received initiator lymphocytes incubated on BP-pulsed macrophages (clear bars) or on macrophages with no antigen (diagonally hatched bars). Other recipients received 10^6 BP-pulsed macrophages (fine stippled bars) or 500 μg of BP directly (coarsely stippled bars). The groups of recruited lymphocytes were incubated without added antigens (control) or with 15 $\mu\text{g ml}^{-1}$ of myelin protein (BP) or lysozyme.

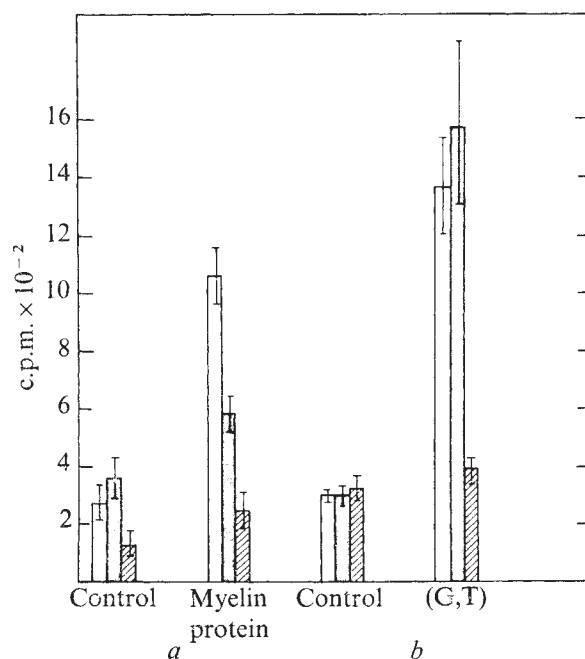
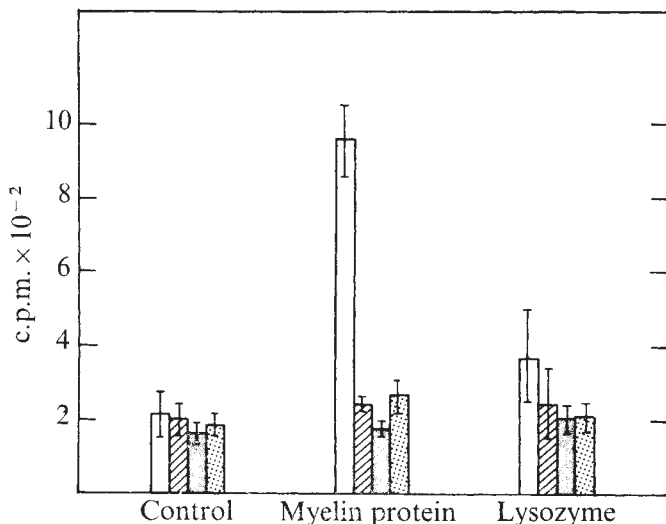


Fig. 3 Blocking of autosensitisation to BP by the presence of soluble BP. Incorporation of ^3H -thymidine was measured in recruited lymphocytes from the draining popliteal lymph nodes of recipients 13 d after injection. Recipient animals (Group a) received 10×10^6 initiator lymphocytes that had been incubated on BP-pulsed macrophages without (clear bars) or with 100 $\mu\text{g ml}^{-1}$ BP in the culture (stippled bars). Other recipients (diagonally hatched bars) received 10^6 BP-pulsed macrophages. In Group b recipients received either 10×10^6 initiator lymphocytes incubated on G,T-pulsed macrophages without (clear bars) or with 100 $\mu\text{g ml}^{-1}$ BP in the culture (stippled bars). Some recipients received 10^6 G,T-pulsed macrophages directly (diagonally hatched bars). The groups of recruited lymphocytes were incubated without added antigens (control) or with 15 $\mu\text{g ml}^{-1}$ of myelin protein (BP) or G,T.

Next, we tested whether soluble BP might inhibit the activation of self-recognising initiator lymphocytes by macrophage-bound BP (Fig. 3). Initiator lymphocytes exposed to BP-pulsed macrophages recruited recipient lymphocytes to respond to BP (SI=3.9). However, when initiator lymphocytes were exposed to BP-pulsed macrophages in the presence of 100 $\mu\text{g ml}^{-1}$ of BP in the culture medium, the response of the recruited lymphocytes was markedly reduced (SI=1.6; $P<0.001$). As above, injection of BP-pulsed macrophages (10^6) by themselves failed to recruit a response to BP (SI=1.8).

The inhibitory effect of soluble BP was found to be specific (Fig. 3). Initiator lymphocytes incubated on syngeneic macrophages pulsed with a copolymer of glutamic acid and tyrosine (G,T), recruited a response to G,T (SI=4.7). The presence of 100 $\mu\text{g ml}^{-1}$ of soluble BP did not inhibit this response to G,T (SI=5.4), indicating that the soluble BP was not toxic to lymphocytes. The control injection of G,T-pulsed macrophages did not recruit a response to G,T (SI=12). Thus, the presence of soluble BP specifically blocked the induction of sensitisation to macrophage-bound BP and not to an unrelated antigen.

We have demonstrated that healthy guinea pigs possess lymphocytes that can recognise and be activated by a normal self-constituent, BP, when it is associated with syngeneic macrophages *in vitro*. These lymphocytes, which at least when *in vitro* possess receptors for guinea pig BP, can recruit immunospecific autosensitised lymphocytes in a syngeneic recipient. Indeed, normal as well as immune lymphocytes were observed to bind myelin basic protein, presumably by way of specific receptors⁷.

In the experiments reported here we did not observe

clinical or pathological signs of EEA in guinea pigs receiving 10^7 initiator lymphocytes. In earlier studies in this laboratory⁸, lesions of EAE were produced in rats by the injection of about 4×10^6 initiator lymphocytes of thymic origin sensitised *in vitro* against a crude extract of rat brain. Thus, the development of overt EAE might require injection of many more than the 10^7 initiator lymphocytes sufficient to recruit a detectable proliferative response to BP. Although the conditions for induction of disease were not met, our results indicate that receptor-bearing lymphocytes can be induced to immune differentiation by BP associated with syngeneic macrophages. Furthermore, the presence of the same BP in soluble form can block the activation of lymphocytes by immunogenic BP.

These findings suggest that the mechanism involved in the encephalitogenic activity *in vivo* of BP injected with adjuvant might include activation of host macrophages to present BP lymphocytes in an immunogenic form. It has been shown that adjuvants stimulate macrophages⁹. However, it is not clear how macrophages might render BP immunogenic for initiator lymphocytes either *in vivo* or *in vitro*. Studies in this laboratory have shown that antigen extracts from syngeneic tumour cells can be immunogenic for mouse lymphocytes *in vitro* when associated with macrophages¹⁰. Thus, macrophages seem to have an important function in regulating the immunogenicity of "weak" self- or tumour-antigens, as well as that of extraneous foreign antigens¹¹.

The blocking of immunogenicity by soluble BP is compatible with the concept that non-immunogenic forms of self-antigens might help maintain natural tolerance to self by blocking lymphocyte receptors and preventing their triggering by immunogenic cell-bound forms of the self-antigens¹². This concept was originally deduced from studies of recognition and triggering of lymphocytes by self-antigens on fibroblasts or thymic reticulum cells^{12,13}. The results presented here strengthen this notion as they were obtained without use of heterologous serum which may have influenced the results of the fibroblast or reticulum cell model systems. The use of purified BP as a self-antigen argues against the possibility that sensitisation was directed against unknown viral or embryonic antigens which might have been expressed in cell culture. Furthermore, induction of autosensitisation in the complete absence of heterologous serum rules out any artefacts resulting from possible sensitisation against foreign antigens present in serum¹⁴. The recent experiments of Wekerle and Begemann, using an *in vitro* model of autosensitisation against testis antigens provides additional support for the concept of blocking self-antigens as regulators of self recognition¹⁵. It remains to be demonstrated, however, that soluble self-antigens operate physiologically *in vivo* to prevent autoimmunity by blocking recognition of the same self-antigens in immunogenic form.

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Naturally-occurring antibodies to poly(ADP-ribose) in patients with systemic lupus erythematosus

POLY(ADP-RIBOSE) is a biopolymer which is synthesised by poly(ADP-ribose) polymerase in cell nuclei¹. Its structure and physicochemical characteristics have been reviewed¹. Its biological function was suggested to be related to DNA synthesis in nuclei²⁻⁴, and there are several reports on its natural occurrence⁵⁻⁹. We reported previously that a specific antibody to poly(ADP-ribose) could be produced in rabbits injected with a complex of poly(ADP-ribose) and methylated bovine serum albumin¹⁰. Kidwell and Mage reported on the presence of poly(ADP-ribose) in HeLa cells and the correlation of its content to cell cycles by a radioimmunoassay¹¹. Recently we found that binding activities of the sera of patients with systemic lupus erythematosus (SLE) to ¹⁴C-poly(ADP-ribose) were higher than those of the sera of normal individuals, and we suggest that poly(ADP-ribose) as well as antibodies to poly(ADP-ribose) occur naturally in human beings. This paper reports that binding activities of the sera of SLE patients to ¹⁴C-poly(ADP-ribose) are attributable to immunoglobulins, mainly to IgG, but not to nonspecific interactions between ¹⁴C-poly(ADP-ribose) and IgG; specificity of the binding was verified by inhibition tests using unlabelled poly(ADP-ribose) and 14 related compounds.

Figure 1 shows binding activities of the sera of SLE patients to ¹⁴C-poly(ADP-ribose) tested by a Millipore filter method, as described in the legend to Fig. 1. The binding of the sera of SLE patients to ¹⁴C-poly(ADP-ribose) was higher than that of the sera of patients with other diseases or normal individuals. It is well known that antibodies to nucleic acids occur with great frequency in SLE patients¹³⁻¹⁵. Poly(ADP-ribose) is a well characterised biopolymer synthesised by a nuclear enzyme¹ and highly immunogenic¹⁰, and thus, we postulated that high binding of SLE sera to ¹⁴C-poly(ADP-ribose) was due to naturally-occurring antibodies to poly(ADP-ribose).

To examine this hypothesis, crude immunoglobulin fractions obtained from five different SLE sera showing high binding activity were applied to a column of Sephadex G-150. As controls, crude immunoglobulin fractions from two sera of different normal individuals were similarly applied to a column. Figure 2 shows profiles of ¹⁴C-poly(ADP-ribose) binding in the fractions. In all SLE sera, ¹⁴C-poly(ADP-ribose) binding activity was observed in the 7S as well as the 19S γ -globulin fractions. On the other hand, in the sera from normal individuals, ¹⁴C-poly(ADP-ribose) binding activity was extremely low and was found only in the 19S γ -globulin fractions.

To prove that ¹⁴C-poly(ADP-ribose) binding in the fraction from SLE patients was attributable to immunoglobulin itself, the double antibody method, using anti- μ and anti- γ chain sera (Behringwerke) was applied to the fraction of Fig. 2a, as follows. Amounts of ¹⁴C-poly(ADP-ribose), and aliquots of the Sephadex fractions used were the same as in the Millipore filter method. After incubation in phosphate-buffered saline-bovine serum albumin at 37 °C

for 1 h, 100 or 200 μ l of anti- μ or anti- γ chain sera was added with 20 μ l of normal human serum as a carrier. The mixture was further incubated at 37 °C for 1 h, and kept at 4 °C overnight. The precipitate thus formed was collected by centrifugation at 4 °C, washed once with cold PBS and solubilised in an NCS tissue solubiliser and the radioactivity was measured in a liquid scintillation counter. Profiles of 14 C-poly(ADP-ribose) binding obtained by the double antibody method coincided very well with those obtained by the Millipore filter method (data not shown).

In addition, F(ab')₂ was isolated from the sera of SLE patients as well as a normal individual, according to the method of Nisonoff *et al.*^{17,18} as described in the legend to Fig. 3. 14 C-poly(ADP-ribose) binding to the F(ab')₂, as a function of their concentrations, is shown in Fig. 3. All the F(ab')₂ from different SLE patients clearly showed 14 C-poly(ADP-ribose) binding, whereas there was practically

Fig. 1 14 C-poly(ADP-ribose) and unlabelled poly(ADP-ribose) were prepared by the method of Sugimura *et al.*¹². The assay of 14 C-poly(ADP-ribose) binding to serum was carried out by a slight modification of the previously described method¹⁰, as follows: A mixture of 14 C-poly(ADP-ribose) (3,500 c.p.m., 4 ng) and 10 μ l of serum was adjusted to a volume of 1 ml with phosphate-buffered saline containing 0.1% bovine serum albumin, pH 7.4 (PBS-BSA), and incubated first at 37 °C for 1 h with gentle shaking followed by 1 h incubation at 0 °C. After incubation, the mixture was poured onto a 0.22- μ m pore size (GS WP 025 00) Millipore filter and washed with 3 ml of phosphate-buffered saline, pH 7.4 (PBS) at room temperature. The Millipore filter was then dried and the radioactivity was measured, using a Toluene-based scintillator by a liquid scintillation counter (Packard). In the absence of serum, the radioactivity trapped on the Millipore filter was less than 5% of the added 14 C-poly(ADP-ribose). The background count was subtracted from all experimental values. The serum was incubated at 56 °C for 30 min before use. *a*, SLE; *b*, rheumatoid arthritis; *c*, patients with other diseases; *d*, normal individuals.

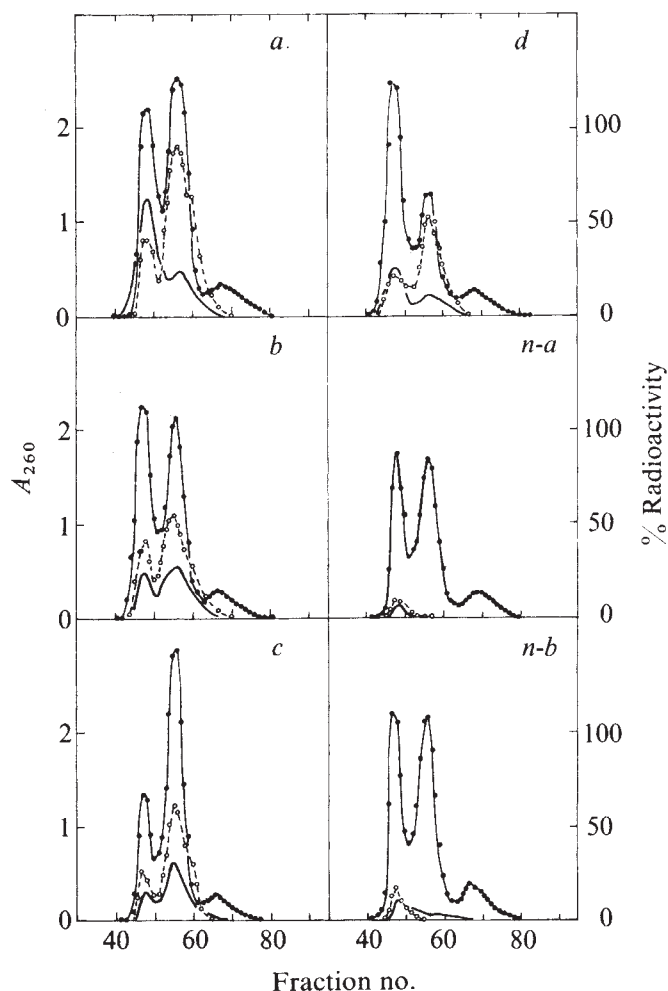
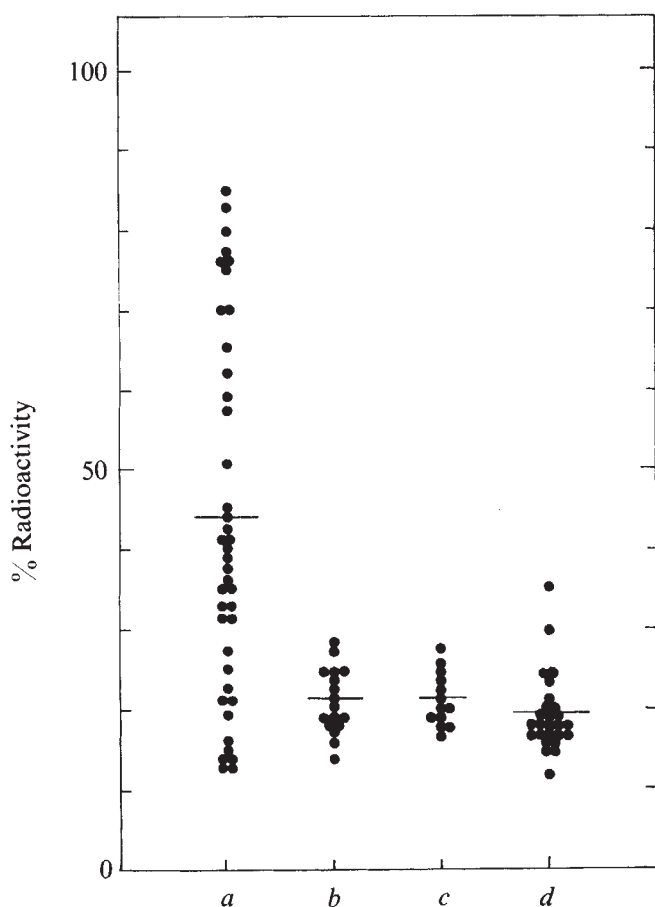


Fig. 2 Sera were diluted twice in PBS and were precipitated in half-saturated ammonium sulphate. The crude γ globulin (1.5 ml) was applied to a column of Sephadex G-150 (1.8 $\times$ 84 cm), which had been equilibrated with PBS. Two-millilitre eluate fractions were collected at a flow rate of 10 ml h⁻¹. An aliquot of each fraction (70 μ l) was used for the assay of the binding to 14 C-poly(ADP-ribose), as described in Fig. 1. Native DNA binding in each fraction was assayed using 3 H-native DNA. 3 H-native DNA (2,700 c.p.m., 75 ng) was prepared by the method of Botchan *et al.*¹⁶ from cultured mouse myeloma cells (L5178Y). Assay conditions were the same as for 14 C-poly(ADP-ribose), described in Fig. 1. Four cases out of five different SLE patients and two normal individuals are presented here. *a*, *b*, *c* and *d*, SLE patients; *n-a* and *n-b*, normal individuals. --- O ---, 14 C-poly(ADP-ribose) binding; —●—, 3 H-native DNA binding.



no binding with F(ab')₂ from the normal individual. These results indicate that the high 14 C-poly(ADP-ribose) binding to the sera of SLE patients is attributable to specific interactions between 14 C-poly(ADP-ribose) and immunoglobulin, mainly IgG.

The specificity of 14 C-poly(ADP-ribose) binding to the 7S γ -globulin fraction, in five cases of SLE sera, was tested by inhibition tests as shown in Table 1. Inhibition of 14 C-poly(ADP-ribose) binding was caused partly by poly(A)-poly(U), poly(A) and poly(I), therefore the 14 C-poly(ADP-ribose) binding to SLE sera may represent a sum of cross reactions with antibodies to these nucleic acid forms. To clarify this, the SLE sera were subjected to absorption experiments with poly(A)-poly(U), poly(A) and poly(I). The residual binding of the 14 C-poly(ADP-ribose) by absorbed sera was no longer inhibited by poly(A)-poly(U), poly(A) and poly(I), but only by unlabelled poly(ADP-ribose), thereby 14 C-poly(ADP-ribose) binding to the SLE sera, shown in Fig. 1, seems

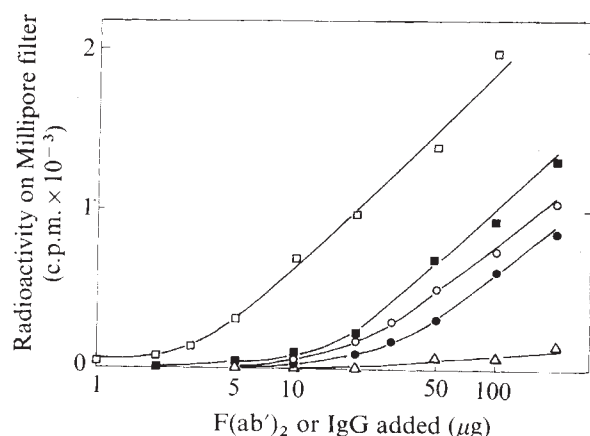


Fig. 3 IgGs of SLE patients *b*, *c*, *d* and normal individual *n-a* were purified from 7S γ -globulin fraction (2nd peak of Fig. 2) by the method described previously¹⁰. They were digested with 10% crystallised hog stomach pepsin (Sigma) in 0.1 M sodium acetate buffer, pH 4.8 at 37 °C for 18 h and neutralised with 1 N NaOH. F(ab')₂, thus formed, were precipitated at a concentration of 18% sodium sulphate. The precipitate was once washed with a solution of 18% sodium sulphate, and was dialysed overnight against PBS. The ¹⁴C-poly(ADP-ribose) binding assay was performed by the Millipore filter method, as described in the Fig. 1 legend. □, untreated IgG from SLE patient *c*; ■, F(ab')₂ from SLE patient *c*; ○, F(ab')₂ from SLE patient *b*; ●, F(ab')₂ from SLE patient *d*; △, F(ab')₂ from normal individual.

to be immunologically specific. Variations in the degree of inhibition, shown in Table 1, may reflect the complexity as well as the state of the disease.

Furthermore, the SLE serum antibodies to poly(ADP-ribose) were most reactive to the poly(ADP-ribose) with 20 repeating ADP-ribose units which was used in the text, and the reactivity of the antibodies was dependent on the chain length of the polymer. This was also true for experimentally induced rabbit antibodies to poly(ADP-ribose).

As native DNA was not a good inhibitor (Table 1) and profiles of ³H-native DNA binding in the fraction of Sephadex G-150 column were different from those of ¹⁴C-

poly(ADP-ribose binding (Fig. 2), antibodies to poly(ADP-ribose) seem to be completely different from antibodies to native DNA. Antibodies to poly(ADP-ribose) in some SLE patients so far examined still maintained the high levels even after reduction in levels of antibodies to native DNA. The value of the serum antibodies to poly(ADP-ribose) for the judgement of clinical state of SLE patients remains to be studied.

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Table 1 Inhibition of ¹⁴C-poly(ADP-ribose) binding to 7S γ -globulin fractions by unlabelled poly(ADP-ribose) and related compounds

Related compound	SLE patient				
	<i>a</i>	<i>b</i>	<i>c</i>	<i>d</i>	<i>e</i>
	% Inhibition				
Poly(ADP-ribose)	82	58	75	55	69
ADP-ribose	5	10	0	10	19
Poly(A)-poly(U)	32	20	14	12	31
Poly(I)-poly(C)	12	6	1	9	17
Poly(A)	25	0	5	2	16
Poly(U)	5	0	4	8	11
Poly(I)	14	0	13	14	19
Calf thymus DNA (native)	4	9	0	7	13
Calf thymus DNA (denatured)	9	0	0	12	11
SV40 viral DNA	0	0	0	4	4
Yeast RNA	12	3	0	7	11
<i>E. coli</i> tRNA	4	0	0	8	10
Rat liver ribosomal RNA	0	4	0	4	6
5'-Adenosine monophosphate	1	3	1	7	14
3'-Adenosine monophosphate	0	0	5	6	6

A 250-fold excess (1 μ g) of unlabelled poly(ADP-ribose), or related compounds, was incubated with 7S γ -globulin fraction of Fig. 2 in the presence of ¹⁴C-poly(ADP-ribose) (3,500 c.p.m., 4 ng), and ¹⁴C-poly(ADP-ribose) binding to the fraction was measured by the Millipore filter method as described in the legend to Fig. 1. In the absence of unlabelled poly(ADP-ribose) or related compounds, samples from SLE patients *a*, *b*, *c*, *d* and *e* were adjusted to bind 65% of the radioactivity added as ¹⁴C-poly(ADP-ribose).

Potassium ion noise currents and inactivation in voltage-clamped node of Ranvier

THE experimental results of voltage noise analyses for the potassium channel system in frog nerve membranes are inconsistent with the theoretical expectations: the measured noise intensity should decrease at positive membrane potentials, where an increase is found^{1,2}. The theories³⁻⁷ are based upon the empirical Hodgkin-Huxley equations, and therefore "... cover only the short term responses of the membrane ..."⁸. Our noise experiments were made in the stationary state. A recent report suggests Hodgkin-Huxley kinetics for potassium noise currents measured earlier after changes in membrane potential⁹. Taken together these results suggest, however, that the potassium channel system changes its behaviour in time with respect to its noise pattern, which implies the occurrence of structural changes in time. We show here that the potassium channel system does indeed change structurally during long duration voltage steps.

The mean membrane current changes in time in response to a pulse of constant voltage (voltage clamp). It rises to a

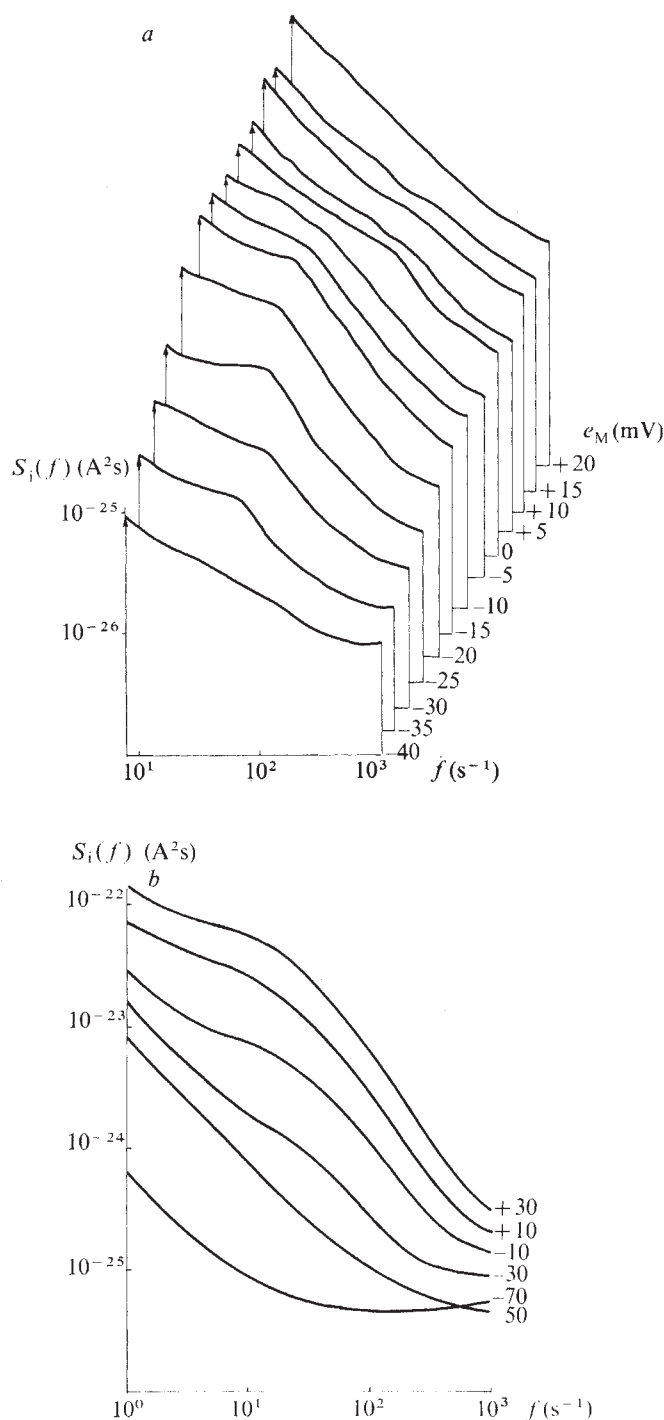


Fig. 1 Spectral densities $S(f)$ of potassium noise currents as a function of membrane potential for one node of Ranvier, at 21 °C. Phase 1 spectra in *a* and phase 2 spectra in *b*. The membrane current was filtered by a two-pole Butterworth high-pass filter with a cutoff at 5 s^{-1} . For phase 1 noise measurements 30–60 voltage steps of 600 ms each were registered per membrane potential. Each signal was further amplified (5,000 times) after a delay of 400 ms to allow the filter transients to sufficiently decay below the membrane noise level¹⁶, and subsequently recorded on analogue tape (bandwidth $0.5 \times 10^3 \text{ s}^{-1}$). After low pass filtering (f^{-8} , Butterworth response) the digitalised correlation function estimator, calculated by a HP3721 correlator, was multiplied with a time window after Hann and Fourier transformed. The set of 30–60 Fourier transforms was averaged for each spectrum. For phase 2 noise measurements single voltage steps of 60–100 s were used at each potential. The measurements were started 20–40 s after the beginning of a step to be in the steady state of inactivation. The spectra were obtained in a similar way, but here the averaged correlation function was used instead.

maximum within 25 ms and stays there for pulses shorter than 200 ms (refs 10, 11). Inactivation, that is, a subsequent decrease of the potassium current, occurs with longer pulses. The decrease proceeds in two phases: a relatively fast decline followed by a slower decrease to a non-zero stationary level¹².

We measured potassium noise currents both during the quasi-stationary early part of the first decline in potassium current (here called phase 1) and in the stationary state after the end of the slow decrease in current (phase 2).

Single sciatic motor fibres of *Rana esculenta* were voltage clamped in a setup after Frankenhaeuser¹³, designed for low noise performance and for measurement of the absolute value of the membrane current¹⁴. The sodium system was blocked with TTX (tetrodotoxin, 300 nM in the bathing medium). The mean potassium current was measured with 25-ms voltage pulses, preceded by a 2-s pulse at -100 mV to remove inactivation. The currents were digitised, stored and subsequently analysed in terms of the Hodgkin–Huxley equations. Phase 1 noise measurements were made with 600-ms duration voltage pulses, with current noise analysis in the interval from 400 to 600 ms (ref 15). 60–100-s voltage steps were used for phase 2 investigations, where the noise analysis was started after complete inactivation.

Both in phase 1 and in phase 2, two noise components were found in the spectra (Fig. 1). A relaxation noise component (the humps in the spectra) and a f^{-1} noise component (with spectral intensity $S(f) = c/f$, slope -1 in the graphs). The relaxation noise component (obtained after correction for the f^{-1} noise¹⁶) has a constant spectral intensity $S(+0)$ at low frequencies and declines with f^{-2} at high frequencies (slope -2). The intersection between the two frequency asymptotes gives the corner frequency f_c .

The potassium channel blocker TEA (10 mM tetraethylammonium chloride in the bath) decreases both components in both phases, as expected^{1,2,9}. The effect of TEA, however, differs for the two phases. The phase 1 noise intensity $S(+0)$ is about 10 times smaller than before TEA application, while the remaining intensity in phase 2 is quite large: at least 70% of its unpoisoned value. The mean potassium current behaves similarly. The initial reduction by 10 mM TEA to about 5% of the original value^{17,18}, is followed, however, by an increase during the period corresponding with the decrease of the potassium current (inactivation) in the unpoisoned state. The final current intensity under TEA is about 50% of its value in the untreated state. The potassium channel system of frog nodes, therefore, changes its properties quite drastically with time.

Investigation of the potassium noise properties confirms this (Fig. 2). The low frequency intensities $S(+0)$ are a function of membrane potential (Fig. 2a) and go through a maximum for phase 1 noise (near -20 mV). $S(+0)$ however, shows a different behaviour in phase 2, in accordance with our previous results². The cutoff frequencies f_c (Fig. 2b) also behave quite differently with a pronounced dependence on membrane voltage⁹ in phase 1 (above -10 mV) but not in phase 2^{2,16} (with $f_c \approx 45 \text{ s}^{-1}$ at 21 °C).

It follows that the potassium relaxation noises in phases 1 and 2 are generated by different sources. Here the frog nerve differs from the squid axon where “phase 2” noise is reported to be related to Hodgkin–Huxley potassium kinetics^{19,20}. In frog nodes phase 1 noise is most probably related to the potassium Hodgkin–Huxley kinetics. We calculated the properties of the potassium relaxation noises according to the simplest translation of the Hodgkin–Huxley equations in stochastic terms^{3,4}, using the Hodgkin–Huxley parameters from the 25-ms voltage clamp experiments. The results (lines in Fig. 2) show that the measured phase 1 intensities $S(+0)$ follow the predicted relation quite well. This parameter may be less sensitive for small deviations from Hodgkin–Huxley kinetics than the kinetic parameter f_c , which shows a deviation. Here partial inactivation present in phase 1, although small, may also have a role. The potassium channel conductance γ_K was cal-

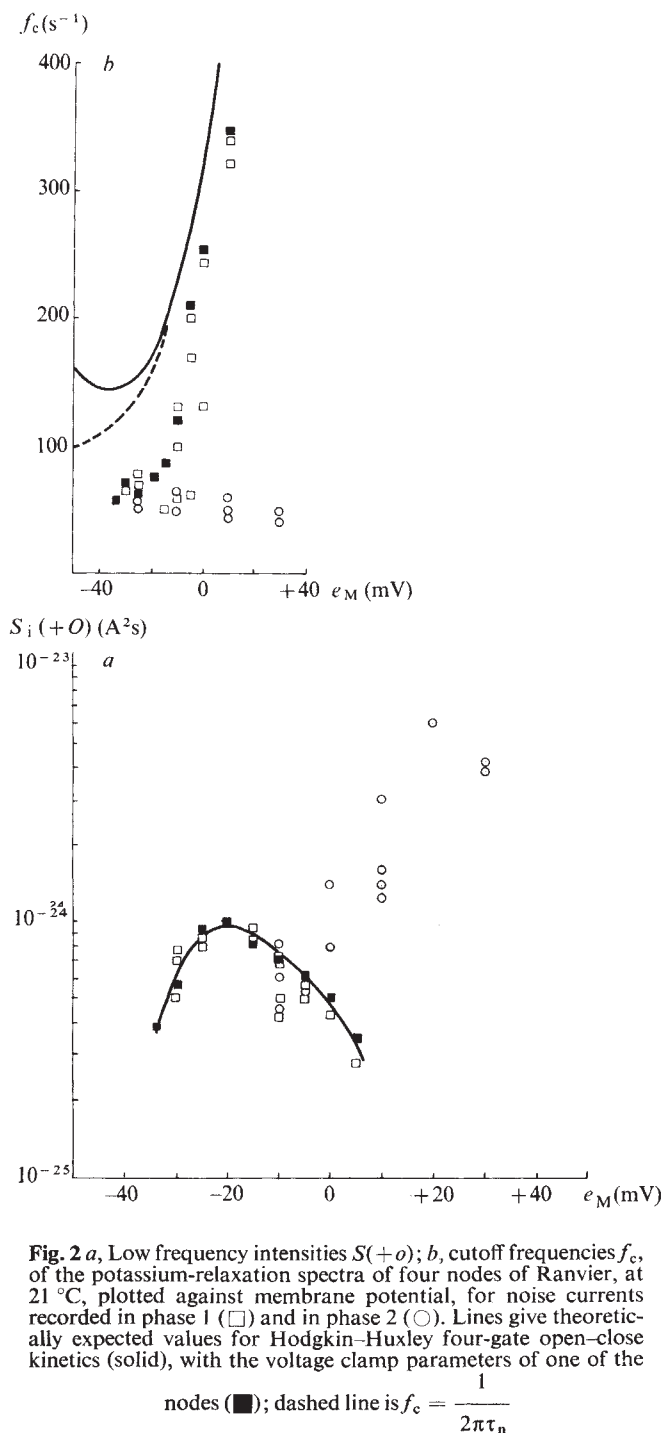


Fig. 2 a, Low frequency intensities $S(+o)$; b, cutoff frequencies f_c , of the potassium-relaxation spectra of four nodes of Ranvier, at 21 °C, plotted against membrane potential, for noise currents recorded in phase 1 ($\square$) and in phase 2 ($\circ$). Lines give theoretically expected values for Hodgkin-Huxley four-gate open-close kinetics (solid), with the voltage clamp parameters of one of the

nodes ($\blacksquare$); dashed line is $f_c = \frac{1}{2\pi\tau_n}$

culated, assuming that all channels are in the open state when the mean potassium conductance $\langle g_K \rangle$ reaches its maximal value $\bar{g}_K$ at a certain positive membrane potential. The channel conductance then follows from^{3,7}:

$$\gamma_K = \frac{\sigma_1^2}{\langle I_K \rangle (E - E_K) (1 - \langle g_K \rangle / \bar{g}_K)} \quad (1)$$

with mean potassium current $\langle I_K \rangle$, membrane potential E and potassium equilibrium potential E_K . The variance of the current fluctuations, σ_1^2 , was obtained by integration of the one-sided spectrum. This gave the estimate for $\gamma_K = 2.9 \pm 0.4$ pS (three nodes, 12 estimates, at 21 °C). This value, which is close to that reported by Begenisich and Stevens⁹ (4.0 ± 0.27 pS, 14

observations at 15 °C) does not depend on membrane potential within its measurable range (-30 to $+5$ mV).

The subsequent change in behaviour must be due to either conformational changes of the channel system or a new potassium channel system arising during inactivation. Which possibility actually occurs must be left open. With the conformational change assumption, however, the channel conductance in phase 2 can be calculated with equation (1). The phase 2 channel conductance now seems to depend strongly on membrane potential, increasing more than twice in the range from -10 to $+30$ mV. This implies that equation (1) does not yield the open channel conductance and necessitates the use of a specific channel model⁷.

Our results prove that the potassium channel system in frog nodal membrane changes its properties profoundly during long duration voltage steps. Another new result, the difference in pharmacological behaviour with regard to the passage of time after the application of a voltage step, shown here to exist for TEA, may also have practical implications. We thank Dr J. de Goede and Professor Dr A. A. Verveen for advice and support and Dr H. D. van Dorp for the computer program. We also thank W. C. Deegenaars, J. H. Kussendrager and A. de Vos for technical assistance. This work was supported by a grant from The Netherlands Organisation for the Advancement of Pure Research (ZWO).

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Bioluminescence of lantern fish (Myctophidae) in response to changes in light intensity

THE characteristic ventral distribution of fish photophores suggests that their luminescence reduces the silhouette when viewed against a background of downwelling light^{1–3}. This hypothesis is supported by demonstration of direct neural control of photophores in several species of myctophids and by morphological and behavioural indications that *Tarletonbenia crenularis* matches its luminescence to background illumination by visually monitoring a supraorbital photophore^{4,5}. There is similar evidence for other taxa: shallow water leiognathids emit light in response to brief photic stimulation and mid-water squid have photosensitive vesicles which may participate in countershading^{6–8}. Here we report photometric measurements of myctophid bioluminescent responses to light levels comparable with those occurring in their environment.

Symbolophorus californiensis (Eigenmann and Eigenmann)

bears approximately 70 photophores of about 0.5 mm diameter on ventral and lateral surfaces. When immature, it lacks the brilliantly flashing luminous patches characteristic of many myctophids. It migrates daily from 600 m to or near the surface⁹. Immature specimens in good condition were netted at night by neuston net from RV Velero IV over the San Clemente Basin. They were transferred to containers of surface temperature seawater and held in darkness for experimentation within the next several hours aboard ship. Individuals were placed in a 1-l cylindrical glass jar in an apparatus with which it was possible to continuously record bioluminescence against controlled background irradiance of up to $0.09 \mu\text{W cm}^{-2}$. Background irradiance was generated by five Sylvania R1166 glow modulator tubes emitting white light onto a diffusing hemisphere above the specimen chamber. An EMI 9781 B photomultiplier, viewing the entire specimen chamber from below, detected bioluminescence. Interference from background irradiance was eliminated by pulsing the glow modulators at 180 Hz in synchrony with a rotating shutter over the photomultiplier tube. The 'on' period for the glow modulators corresponded to the period during which the photomultiplier tube was covered by the shutter. The photomultiplier signal was processed by a Kiethley 840 lock-in amplifier and displayed on a chart recorder with a bandwidth of 70 Hz. Background irradiance was measured with a United Detector Technology 40X radiometer. Tests of the performance of the apparatus showed that a photophore-sized light source, placed 10 cm from the photomultiplier, and producing an estimated irradiance of $5 \times 10^{-5} \mu\text{W cm}^{-2}$, could readily be detected against a 0.5-m^2 background producing an irradiance of $8 \times 10^{-2} \mu\text{W cm}^{-2}$. Because the 180-Hz glow modulator cycle exceeds the highest visual flicker fusion frequency reported for fish, the apparatus makes it possible for a subject to be exposed to perceptually continuous light while being monitored by a photo-

multiplier protected from dark current and sensitivity changes caused by excessive light exposure of the photocathode¹⁰.

Six *S. Californiensis*, 26–30 mm standard length, responded similarly to repeated exposures to various background light regimes. Latency of bioluminescence initiation after switching on background lighting was as little as 0.5 s, with averages for four fish being 18.5 (four tests), 7.0 (one), 1.0 (four) and 2.0 s (six). 'Off' responses were similarly rapid but the data cannot be used for a precise measurement. In many instances a probable luminescent startle response of 0.5–1.0 s latency, requiring an average of 4 s from half amplitude on to half amplitude off, precede initiation of sustained bioluminescence (Fig. 1a and b). Our data cannot be used to estimate how closely the fish match the background irradiance with their photophore emission because the position of the fish is indeterminate. The level of bioluminescence, however, is clearly directly related to background irradiance over part of the background irradiance range. When exposed to a ramp of increasing irradiance, ranging from near 0 to $0.008 \mu\text{W cm}^{-2}$ over 55 s, bioluminescence also increased in ramp-like fashion (Fig. 1a). Assuming a nominal position of 10 cm from the photomultiplier and maximum exposure of the ventral surface of the fish to the photomultiplier, point A in Fig. 1a corresponds to an irradiance of $2 \times 10^{-5} \mu\text{W cm}^{-2}$ at the photocathode of the photomultiplier tube. Further evidence of proportionality of bioluminescence to background light intensity is seen in Fig. 1b and c where bioluminescence is off scale at $0.05 \mu\text{W cm}^{-2}$ background and considerably less at $0.002 \mu\text{W cm}^{-2}$ background. Bioluminescence is inhibited when the background reaches about $0.09 \mu\text{W cm}^{-2}$ and reappears when the background is reduced to $0.02 \mu\text{W cm}^{-2}$, an effect that may be due to visual light adaptation because bioluminescence can be maintained to $0.08 \mu\text{W cm}^{-2}$ background irradiance when the background is cycled from dim to bright.

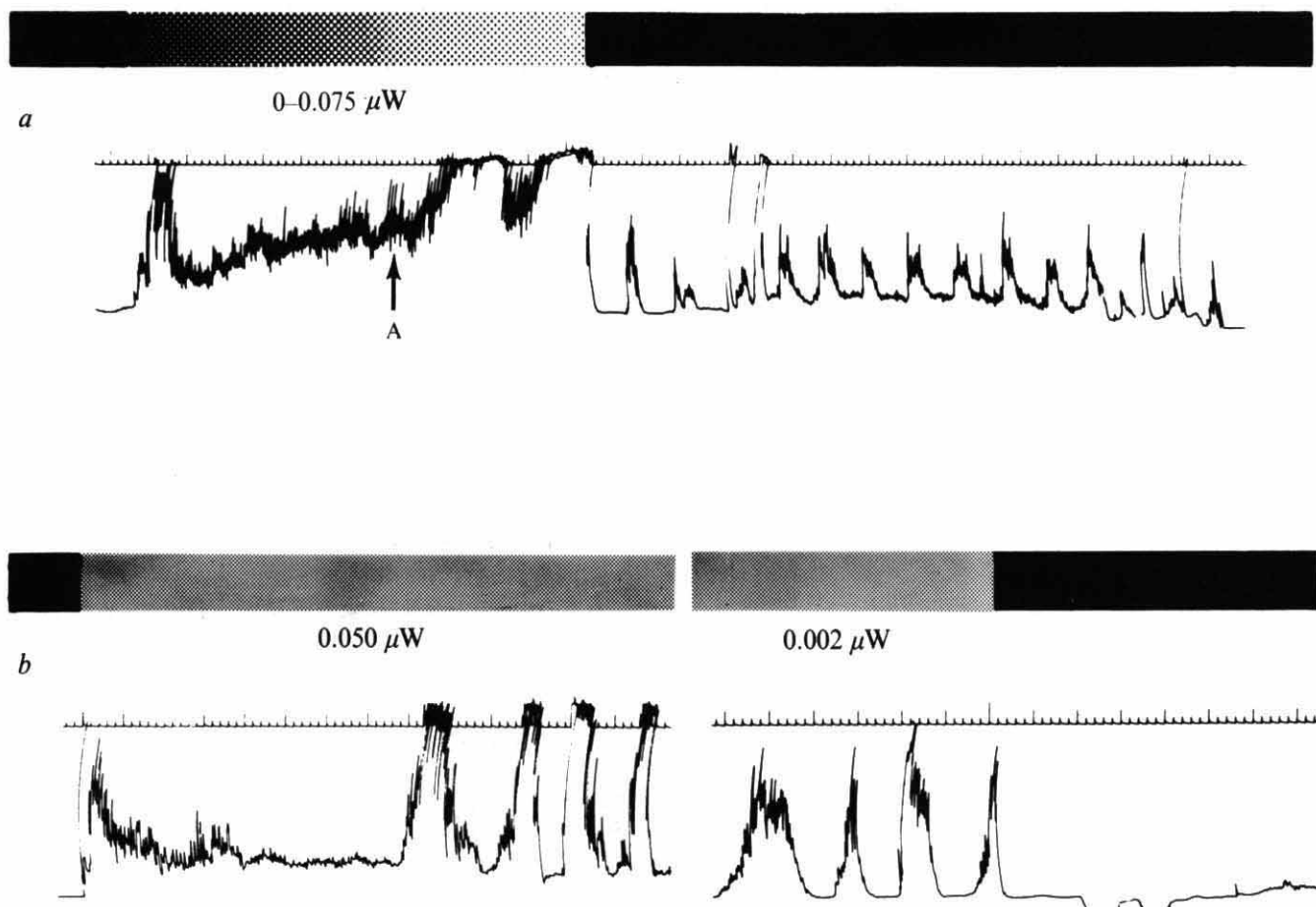


Fig. 1 Photomultiplier recordings of *Symbolophorus* bioluminescent responses to variation in background illumination. a, Probable alarm response and gradually increased bioluminescence in response to gradual increase in background illuminance, followed by diminished bioluminescence in darkness (point A equals $2 \times 10^{-5} \mu\text{W cm}^{-2}$ at photomultiplier); b, probable alarm response followed by delayed onset of presumed countershading bioluminescence on sudden increase in background illuminance; c, sudden cessation of luminescence on cessation of low-level illumination. Records (b) and (c) were made with the same specimen. Time marks at 1 and 5 s, increasing bioluminescence indicated by upward deflection of traces.

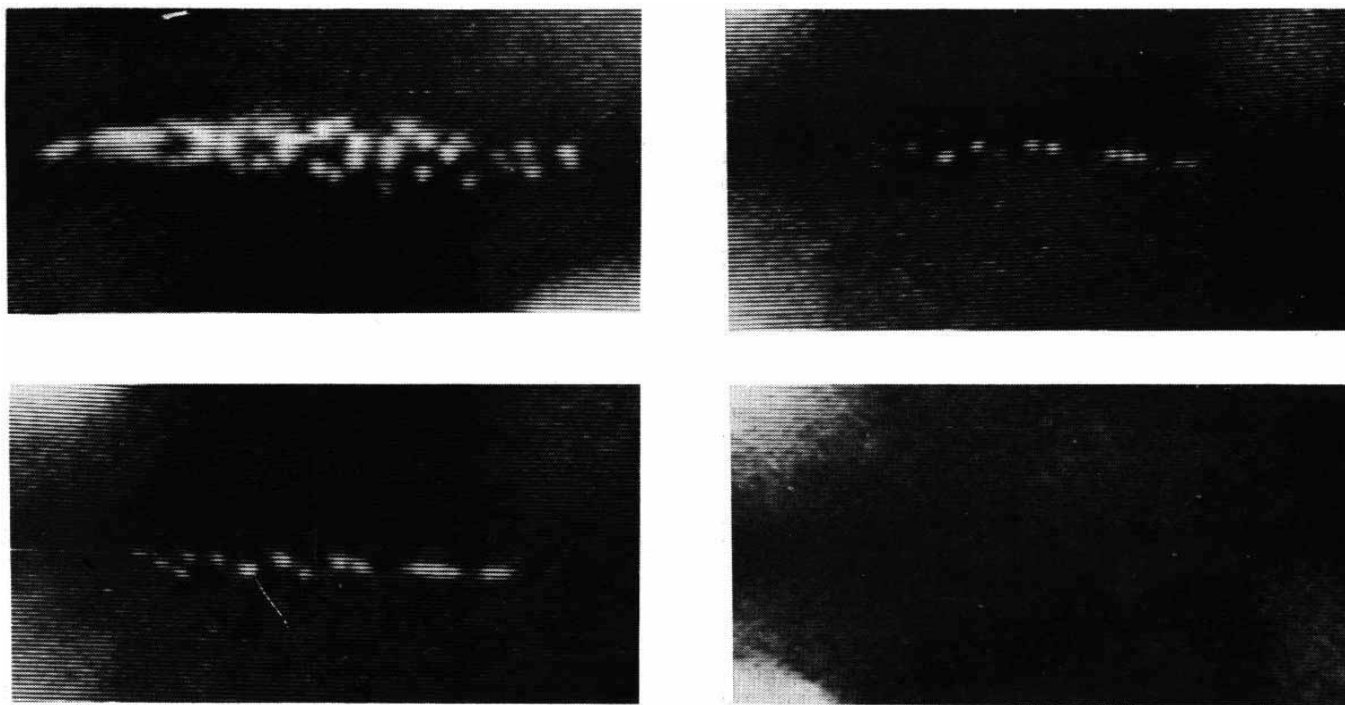


Fig. 2 Image intensified video recordings showing rapid onset of luminescence from ventral photophores of an approximately 5-cm standard length, unidentified myctophid. Interval between first and third frames is 116 ms, 676 ms for the entire series.

During continuous background exposure, bioluminescence continues for at least 15 min, our longest test at a single background irradiance level. The short term peaks (roughly six to eight per min) coincide with the ship's motion. We believe they represent rotation of the free-swimming specimen with reference to the photomultiplier and that bioluminescence during exposure to light is uniform. A similar effect can be produced with an artificial light source in place of the fish. Photomultiplier records (Fig. 1a) and visual observations show some low level bioluminescence in darkness, often due only to the slow winking on and off of a single photophore.

Behaviourally-induced photophore responses of myctophids may be more rapid than the 0.5 s measured with the relatively poor time resolution of our recording system, as suggested in image intensified video recordings made according to the method of Barnes and Case⁴. Figure 2 shows video frames of an unidentified myctophid obtained by trawling in the Banda Sea. Within 677 ms all photophores in view have markedly increased their output.

These experiments demonstrate the presence of the requisite visual sensitivity linked with photophore control to facilitate bioluminescent reduction of the silhouette (countershading or counterillumination) in the typical light regime of lantern fish. Both the rapidity of photophore control and the demonstration of what may be a transient alarm response, however, argue that myctophid photophores have other roles.

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Voltage signal of photoreceptors at visual threshold

It has long been known that vertebrate photoreceptors can signal the absorption of single quanta of light¹. The extraordinary sensitivity of the visual system is usually ascribed both to the sensitivity of the photoreceptors and to the integration of their signals by higher-order neurones. A more complete explanation of visual sensitivity could be given if it were possible to record the response of receptors at the visual threshold. Unfortunately, it has not been possible to do this at the absolute threshold, largely because of interactions between receptors². Near the absolute threshold only a small proportion of the receptors absorb quanta, but the photocurrent from these cells spreads through electronic synapses³ to many other cells. Thus the voltage responses in the receptors are non-uniform, and it is not possible to tell which responses contribute to the threshold. Fortunately this difficulty does not arise at the increment threshold since in the presence of bright background light, the variation in response from one receptor to the next is minimal. The differences among the receptors in the number of quanta caught will be much less important than at absolute threshold, and the connections between the receptors will tend to equalise their voltages. In this report we compare receptor responses to behavioural responses in the light-adapted turtle and show that the receptor response at threshold is only a few microvolts in amplitude.

The amplitude of turtle receptor responses to flashes of light in the presence of bright backgrounds can be estimated from the results of Baylor and Hodgkin⁴. The sensitivity of red-sensitive cones in the turtle *Pseudemys scripta elegans* varies with the intensity of background light according to the relation

$$\frac{1}{S_F} = \frac{1}{S_F^0} + \zeta I_B \quad (1)$$

where S_F is the sensitivity of the cones to brief, increment flashes, S_F^D is the cone sensitivity in the dark (that is, with no background light), I_B is the intensity of the background, and ζ is a parameter having the nearly constant value of 20 s V^{-1} per flash. Near threshold the peak amplitude of the receptor response is equal to the product of the increment flash intensity and S_F , the flash sensitivity⁴⁻⁶. Thus the voltage signal (ΔV_T) at the increment threshold (ΔI_T) is given by

$$\Delta V_T = S_F \Delta I_T \quad (2)$$

As long as ΔV_T is invariant with background intensity, equations (1) and (2) can be combined to give the Weber-Fechner relation for the behavioural threshold

$$\Delta I_T = k(I_B + I_0) \quad (3)$$

with I_0 equal to $\Delta V_T / k S_F^D$ and the Weber fraction, k , equal to $\Delta V_T \zeta$. Since turtle vision has been shown to obey the Weber-Fechner relation^{7,8}, a determination of k can be combined with the previously determined value for ζ to give an estimate of ΔV_T .

To estimate the amplitude of ΔV_T , we have measured increment thresholds for *Pseudemys scripta elegans*, the same species of turtle used by Baylor and Hodgkin^{4,5}. The apparatus has been described previously⁹, but there are some alterations to be noted. The test target consisted of a circular piece of milk glass 4.9 mm in diameter, illuminated by tungsten light from a d.c. source. The test target subtended a visual field of $2^\circ 21'$ and produced an image diameter size of nearly 300 μm on the retina, as estimated from a schematic eye developed for this animal by D. P. M. Northmore (personal communication). The unattenuated intensity of the white test light was measured with a calibrated thermopile to be 13.0 $W m^{-2}$. Background light was afforded by a second lamp similar to the test lamp mounted outside the rear of the chamber, whose unattenuated intensity was 177.5 $W m^{-2}$.

The procedure required a head withdrawal response in order to avoid electric shock. The experimental sequence was under programmed control, and details of training and testing have been reported previously⁹. Stimuli were presented at one minute intervals against a dark background and backgrounds of varying intensity levels. Thresholds were determined by a tracking method such that a response on any trial caused the next stimulus to be reduced in intensity by 0.1 logarithmic unit, and the absence of response caused a like increase in intensity. Determined thresholds were very reliable: The calculated mean 95% confidence interval over the series of thresholds collected was only ± 0.08 logarithmic unit.

The results of the increment threshold measurements are shown in Fig. 1. The data have been fitted with equation (3), using a value for k of 10^{-4} s per flash. Since in light-adapted *Pseudemys* the spectral sensitivity of the behavioural response which we have used for our measurements is in close agreement with the spectral sensitivity of the red-sensitive cones^{5,9} we will assume that the curve in Fig. 1 reflects primarily the activity of the long-wavelength cone system. It is doubtful that green- or blue-sensitive cones contributed to our measurements, since the white light of the test and adapting beams had most of its energy in the red region of the spectrum. In any case, small contributions from green-sensitive cones would not affect the calculation of ΔV_T , since ζ is approximately the same for both green- and red-sensitive receptors⁴.

From the values of k and ζ , we calculate that ΔV_T is approximately 5 μV . Although there is some variability in our measurements, we estimate that this value is accurate to within 0.3 log unit, so that ΔV_T is at most 10 μV . There are several reasons to believe that this value is an upper limit for the threshold signal. First, the target used in the behavioural measurements was larger than that used by Baylor and Hodgkin⁴ and probably produced significant responses in horizontal cells¹⁰. Horizontal cells in turtle inhibit cones¹¹, and it is possible that this inhibition

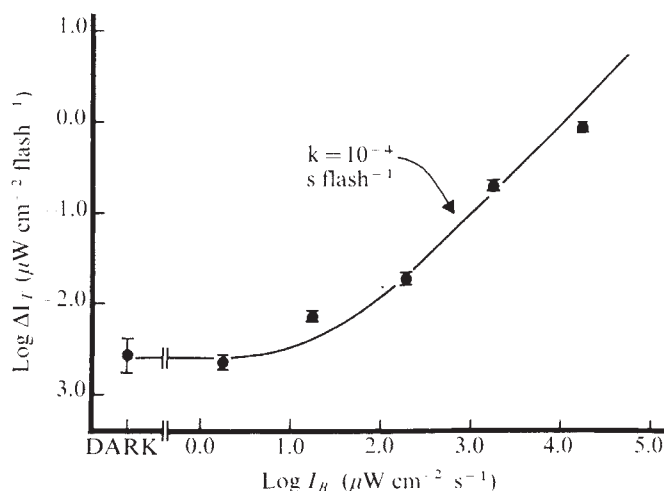


Fig. 1 Increment-threshold curve for the behavioural response of the turtle, *Pseudemys scripta elegans*. Thresholds measured with flashes between 10 and 50 ms have been corrected for the difference in flash lengths and averaged together, since the behavioural threshold in turtle shows complete temporal summation for flashes up to at least that level (Granda and Maxwell, unpublished data). Data points give the mean threshold and bars, one standard deviation. Points have been fitted with equation (3) using a value for k of 10^{-4} s per flash.

was large enough in the behavioural measurements to increase k , thus overestimating ΔV_T . Second, we do not know that our test target was optimum for eliciting responses from turtles. Finally, the intracellular measurements of cone responses were most probably made in the nuclear region of the cell body. The voltage in the synaptic pedicle is probably somewhat smaller.

Our results show that a receptor response of no more than 10 μV is sufficient to signal visual detection. This means that a very small voltage can change the flow of synaptic transmitter between receptors and second-order neurones, suggesting that the sensitivity of transmission at the receptor synapse makes an important contribution to the sensitivity of photodetection. It is of some interest that the threshold signal in photoreceptors appears to be comparable in magnitude to the signals estimated to produce threshold responses in electroreceptors¹². Both photoreceptors and electroreceptors make synapses with second-order neurones which are characterized by presynaptic specializations including a synaptic ribbon or bar and an arciform or presynaptic density¹³ which may function to direct the release of synaptic transmitter^{14,15}. The similarity in the microanatomy of the synapses in these two systems suggests that the characteristic presynaptic specialisation at receptor synapses may serve to permit the signalling of small amplitude responses.

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Unusual resistance to ultraviolet light in dark phase of blue-green bacterium *Anacystis nidulans*

THE role of visible light in the repair of ultraviolet light-induced damage has been extensively studied in various organisms¹. In blue-green bacteria, photoreactivation has been found to be extremely efficient². Indeed, in these organisms, which generally need light as a growth factor, the presence of an efficient photoreactivation mechanism hinders the detection of alternative repair steps which may be present. In spite of this difficulty, however, the existence of light-independent (that is, dark) repair has been observed in blue-green bacteria as suggested by the isolation of mutants with altered ultraviolet light sensitivity^{3,4}. Here we report an interesting observation suggesting the presence of a very efficient repair system against damage induced by ultraviolet light (254 nm) in an obligate photoautotrophic blue-green bacterium *Anacystis nidulans*. We show that this repair system is either inhibited or less effective under aerobic conditions in the presence of light.

Synthetic medium, C-Mn, both for liquid and agar plate, was used for growth of *Anacystis nidulans* as described by Van Baalen⁵. Liquid cultures were grown at $36 \pm 0.5^\circ\text{C}$ in a water bath shaker and at a light intensity of 200 foot candles from tungsten lamps. In the exponential phase, the doubling time under these conditions of growth was 5 h. Agar plates with cells spread by glass rod were incubated in tungsten light at an intensity of $200 \pm 10\text{ ft cd}$ and at $35 \pm 1^\circ\text{C}$ for 5 to 6 d after which colony counts were taken. Under these conditions of growth 100% plating efficiency was obtained.

Cells preincubated in the dark acquired a high resistance to ultraviolet light as compared to cells kept in the light (Fig. 1). This was observable in conditions when the repair by photoreactivation was reduced to minimum. It has been shown that after about 18 h of dark incubation following ultraviolet light exposure no significant change in survival can be effected by photoreactivating light, indicating the presence of only insignificant photoreactivable damage⁴. In order to minimise the repair by photoreactivation, we therefore maintained 24 h of post-exposure dark incubation at room temperature (25°C). Since photorespiration is blocked in dark incubated cells we thought it would be interesting to see if blocking the total respiration could change the sensitivity⁶. Indeed, when the cells were incubated in light in anaerobic conditions (that is, in an argon atmosphere), the ultraviolet resistance was restored considerably in them (Fig. 1, curve L_1).

Significant metabolic differences have been found to occur in light and dark phases of *Anacystis nidulans*. In their studies on macromolecular synthesis, Hayashi *et al.* showed that all species of RNA synthesis continued for at least 4 h in the dark in contrast to DNA synthesis which is stopped in the dark⁷. It is also interesting to note that in the dark this organism accumulates relatively

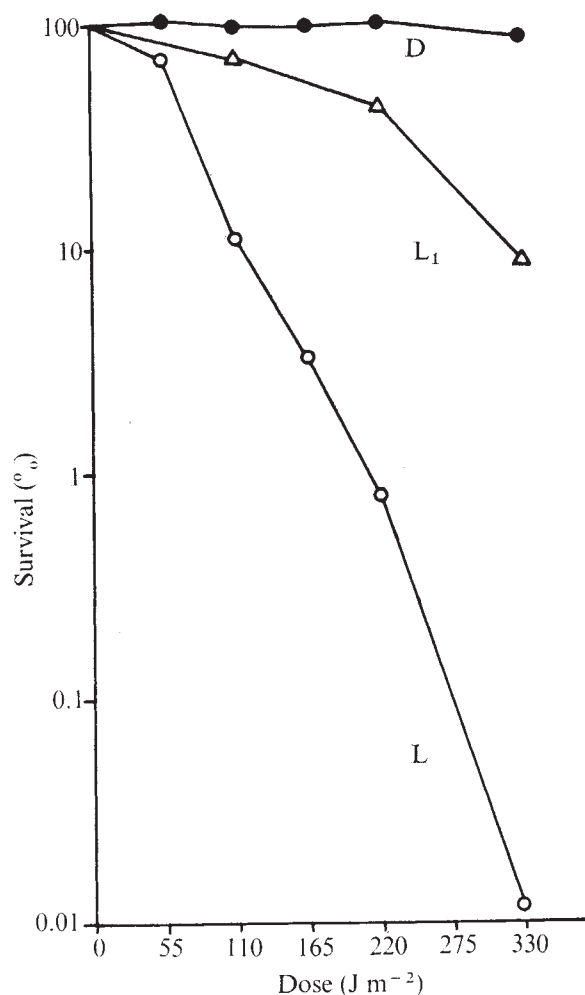


Fig. 1 Survival by *Anacystis nidulans* of exposure to ultraviolet light. Exponential phase cultures of *Anacystis nidulans* were washed with nitrate-free C-Mn medium and resuspended in the same medium at $10^7 - 10^8$ cells ml^{-1} . The suspension was divided into three fractions. One fraction was kept in the dark by covering the flask with aluminium foil (D). The second fraction was kept in a vial with rubber cap, sealed with wax and was sparged with argon gas for 30 min (L_1). The third fraction was kept in an uncovered flask (L). All the samples were transferred within 40 min of washing to a water bath shaker at $36 \pm 0.5^\circ\text{C}$. L and L_1 received tungsten light at an intensity of 200 foot candles. After 24 h of incubation the cells from L, L_1 and D samples were diluted appropriately in C-Mn nitrate-free medium and spread on synthetic agar plates with a sterile glass rod under dim light. Plates with cells were exposed to ultraviolet light (254 nm) from Philips TUV 15 W germicidal lamp at an intensity of $11\text{ J m}^{-2}\text{ s}^{-1}$ as measured by an Eppley 12 junction linear thermopile. The control and the treated plates were kept in the dark for 24 h at room temperature (25°C). After the dark incubation all the plates were transferred to light ($200 \pm 10\text{ ft cd}$) at $35 \pm 1^\circ\text{C}$. At the end of incubation in light for 5-6 d the colony counts were taken.

larger amounts of two stable RNA species whose biological functions are still unknown⁸. These are also synthesised in the illuminated cells but are unstable in them. We have observed that when dark incubated cells are transferred to light the ultraviolet resistance is lost in about 1 h, a period within which these stable RNA species have been reported to disappear in light⁸. We have also observed that dark incubated cells when exposed to visible light immediately after ultraviolet light treatment are more sensitive than those not receiving the visible light exposure. This dose of visible light, however, causes the recovery of a very significant fraction of cells exposed to ultraviolet light which are preincubated in light in aerobic conditions and are extremely sensitive to ultraviolet light (our unpublished data). In view of these

observations we suggest the existence of certain repair steps which are either more efficient in the dark or inhibited in the light under aerobic condition prior to ultraviolet light exposure. The possibility that this differential ultraviolet light sensitivity is due to differences in the nature and extent of primary damage in the light- and dark-incubated cells cannot be totally ruled out by these observations. That this effect is due to the DNA replication inhibition in the dark incubated cells seems improbable. This is because in our experiments both light- and dark-incubated cells were suspended in a nitrate-free medium for 24 h before ultraviolet light exposure. *Anacystis nidulans*, not being a nitrogen-fixing organism, does not grow in the absence of nitrate.

The presence of any repair system against damage induced by ultraviolet light which may either be induced in the dark or inhibited in light is not known. The reverse has, however, been reported. It has been shown that a large, free-living protozoan *Amoeba proteus*, grown in the dark, acquires sensitivity in its G₂ phase to near ultraviolet (365 nm) light as compared with cells cultured in 12 h light/12 h dark cycle⁹. Resistance is restored in dark-grown cells within 10 d of transfer to light/dark cycle. It has also been shown that the *Nicotiana tabacum* plant kept in dark for 7 d lose their capacity to photoreactivate tobacco mosaic virus RNA treated with ultraviolet light¹⁰. This capacity can be restored by transferring the plant to light.

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Evidence for nuclear antigens in cytomegalovirus-transformed human cells

THE anti-complement immunofluorescence (ACIF) method has revealed an antigen localised in the nucleus of both producer and nonproducer cultures of lymphoblastoid cells carrying the Epstein-Barr virus (EBV) genome¹. Early nuclear antigens resembling the Epstein-Barr nuclear antigen (EBNA) have been detected in human embryo lung (HEL) cells as early as 3 h after infection with human cytomegalovirus (CMV)². These early antigens could be detected only by ACIF staining. We have reported³ that hamster embryo fibroblasts (HEF) transformed with ultraviolet-inactivated CMV exhibit cytoplasmic and perinuclear CMV-specific antigens when tested with CMV-immune sera in the indirect immunofluorescence (IF) test. We later noted that a human CMV of genital origin established a long-term persistent infection in HEL cells *in vitro*,

which resulted in the development of two transformed cell lines after several cell culture passages⁴. CMV-specific antigens were demonstrated on the surface of the cells by the indirect IF technique. We have now investigated whether EBNA-like nuclear antigens can be detected in the CMV-transformed human cells with the ACIF technique, and whether there is a correlation of reactivity with early antigens of CMV-infected cells.

Monolayers of HEL cells were grown on coverslips in Dulbecco's medium supplemented with 10% foetal calf serum. The CMV-transformed human cell lines CMV-Mj-HEL-1 and CMV-Mj-HEL-2 and the tumour cell line CMV-Mj-HEL-2, T-1 were established as before (ref. 4 and our unpublished work with J. Kreider). These cells were grown on coverslips in Ham's medium supplemented with 20% foetal calf serum, 0.075% sodium bicarbonate, penicillin (100 IU ml⁻¹) and streptomycin (100 µm ml⁻¹). The C-87 strain of CMV was used for the inoculation of HEL cells at a multiplicity of 0.3 plaque-forming units (PFU) per cell. Cell sheets were fixed 3, 6 and 48 h after inoculation as described before². As a control, the HIL strain of herpes simplex virus type 1 (HSV-1) and the 333 strain of herpes simplex virus type 2 (HSV-2) were used at a multiplicity of infection of 6.0 PFU per cell. Cell sheets were fixed with acetone-methanol 6 h after infection.

Human sera were obtained from hospital patients and staff members of our department. Sera of patients with CMV mononucleosis were supplied by Dr W. Henle of Children's Hospital, Philadelphia. HSV-2-specific hamster serum had an IF antibody titre to HSV-2-infected cells of 1:32.

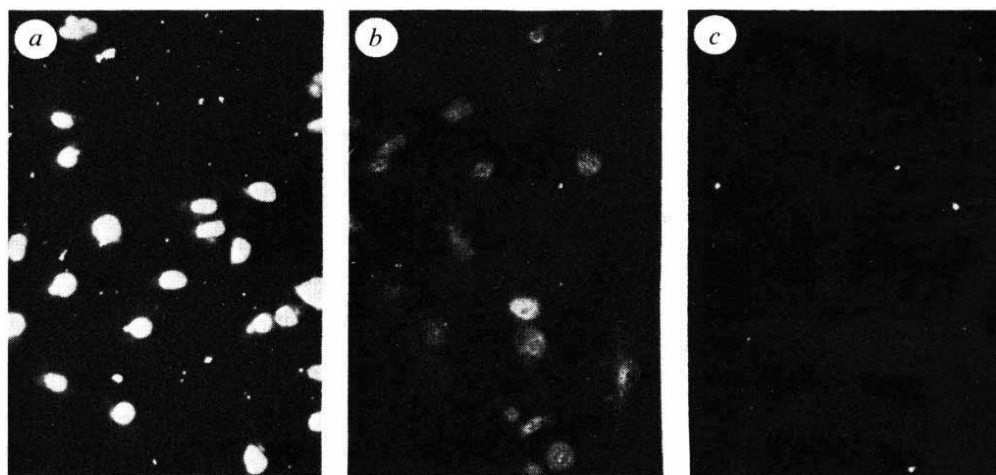
HEL cells, CMV-infected HEL cells or CMV-Mj-HEL-1 cells (10⁶ per ml) were used for the adsorption of the sera. Cells were added to undiluted sera, sonicated and incubated for 1 h at 37 °C and then overnight at 4 °C. The cell debris was removed by high-speed centrifugation.

Anti-human and anti-hamster immunoglobulin G fluorescent conjugate (goat) and fluorescent conjugates for anti-human complement staining (goat) were purchased from Cappel Laboratories, Downingtown, Pennsylvania. Fluorescein isothiocyanate conjugates were adsorbed with 10⁶ target cells per ml before use. CMV-negative and HSV-negative human sera were used as the source of complement in the ACIF tests. Standard procedures² were used for IF and ACIF tests.

The following results were obtained. Eight of thirteen CMV-immune human sera reacted with nuclear antigens of CMV-Mj-HEL-1 cells. Depending on the immune serum used, 6–80% of the cells demonstrated nuclear antigens (Table 1). The reaction observed was intense and located entirely within the nuclei (Fig. 1a). The intensity varied from test to test and among cells within a test, and thus may have depended on the stage of the cell cycle. Occasionally, faint nuclear fluorescence was detected in transformed cells stained by the indirect IF method (Fig. 1b), where a more granular pattern was observed. The immune serum did not react with control HEL cells. The CMV-negative human serum, which was the source of the complement in these tests, did not react with the transformed cells (Fig. 1c).

There was a correlation between the CMV antibody titre of the immune sera and their reactivity with the nuclear antigens; CMV-negative human sera did not react with the nuclei of the CMV-transformed cells (Table 1). All immune sera with antibody titre higher than 1:64 against CMV-infected cells reacted with nuclear antigens of the transformed cells (Table 1). None of the nuclear-antigen-reactive sera reacted with nuclear antigens of control HEL or bladder cancer cells (Table 1). The antibodies to the nuclear antigens of CMV-transformed cells sometimes seemed to develop in the convalescent phase of the infection in some

Fig. 1 *a*, Nuclear antigens in CMV-Mj-HEL-1 cells. CMV-immune human convalescent serum was used with human complement and fluorescein isothiocyanate-conjugated anti-human complement goat serum in an ACIF test. *b*, Nuclear antigens in CMV-Mj-HEL-1 cells after reaction with CMV-specific human convalescent serum and fluorescein isothiocyanate-conjugated goat anti-human immunoglobulin G. Note faint staining of antigens with the indirect IF method. *c*, CMV-Mj-HEL-1 cells were reacted with negative human serum used as a source of complement and fluorescein isothiocyanate-conjugated anti-human complement goat serum in an ACIF test ($\times 500$).



cases of CMV mononucleosis (Table 1, see sera 2 and 3). All sera that reacted with the nuclear antigens of the transformed cells also reacted with the early (6-h) antigens of CMV-infected cells. Only eight of 13 'early-antigen-reactive' immune sera, however, reacted with nuclear formed cells in the ACIF test.

Adsorption of serum with uninfected HEL cells did not influence the reactivity of the serum with the nuclear antigens of CMV-transformed cells or CMV-infected HEL cells. When adsorbed with CMV-infected HEL cells, however, the immune serum lost its reactivity against the nuclei of transformed and virus-infected cells. The reactivity with HSV-infected cells was not influenced. The adsorption of the CMV-immune serum with the CMV-transformed human cells removed the reactivity with the 'early' antigens of the CMV-infected HEL cells and decreased the reactivity with the CMV-infected cells fixed 48 h after infection. HSV-2-immune hamster serum did not react with the nuclear antigens. Sera that were positive for EBNA did not react with CMV-infected or CMV-transformed cells in the ACIF test.

Some of the immune sera reacted with both nuclear and perinuclear antigens, or with only nuclear or perinuclear

antigens in the transformed cells. The perinuclear reaction requires further study.

Thus intranuclear CMV-related antigens have been demonstrated in CMV-transformed human cells by means of the sensitive ACIF method. The staining of the nuclei of the transformed cells by ACIF correlated with the presence in the sera of CMV antibodies against late-phase intracellular and 'early' intranuclear antigens of CMV-infected cells. Not all immune sera with anti-CMV antibodies, however, reacted with the transformed cells. This is not surprising in view of the numerous CMV antigens that may elicit the immune response in man. The nuclear reactivity seems to be possessed primarily by immune sera, taken from patients with prostatic cancer or those in the convalescent phase of an acute CMV infection, with a CMV antibody titre higher than 1:64 as measured by the direct IF test. There is apparently a relationship between the nuclear antigens of the transformed cells and the 'early' 6-h nuclear antigens of CMV-infected cells. Thus, the nuclear antigens of the transformed cells may be CMV-specific T antigens of CMV-transformed human cells and may be important in immunological studies of the role of CMV in human malignancies. This study was supported

Table 1 Reactions of CMV-positive and CMV-negative human sera to ACIF* tests with antigens of CMV-infected and CMV-transformed cells

Serum no.	Diagnosis	IF antibody titre against CMV-infected HEL cells (48 h)	ACIF nuclear reaction with HEL cells	ACIF nuclear reaction with CMV-infected cells (6 h)	ACIF nuclear reaction with CMV-Mj-HEL-1 cells
1	CMV-M†	1/10	—	+	80‡
2	CMV-M§	1/32	—	+	—
3	CMV-M¶	1/32	—	+	23
4	CMV-M§	1/4	—	+	—
5	CMV-M¶	1/64	—	+	—
6	CMV-M§	1/256	—	+	6
7	CMV-M¶	1/256	—	+	9
8	CMV-M§	1/128	—	+	21
9	CMV-M¶	1/128	—	+	21
10	CMV-M§	1/32	—	+	—
11	CMV-M¶	1/32	—	+	—
12	PCa**	1/256	—	+	10–20
13	PCa	1/256	—	+	25–30
14	Normal	< 1/4	—	—	—
15	Normal	< 1/4	—	—	—

*Anti-complement immunofluorescence.

†CMV mononucleosis.

‡Percentage of fluorescing cells.

§Acute phase serum.

¶Matched convalescent phase serum.

**Prostatic cancer.

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Radiation-induced nondisjunction in oocytes of aged mice

SOME human epidemiological studies^{1–4} suggest that there may be an association between nondisjunction and pre-conception exposure to abdominal X rays in women. This response to radiation appears to increase with age^{2,4}. To test this hypothesis, experimental evidence was sought by examining second metaphase chromosomes of cultured oocytes of mice. In the previous experiment⁵, abnormal segregation was induced in young mice, aged 3–6 months,

placed on a slide and all were accounted for during the analyses. All slides were coded to avoid bias.

After analysis of the preparations by Giemsa staining, coverslips were removed by holding the slides under warm running tap water. The preparations were then air dried. C-banding, not used in the previous experiment, was used when the chromosome count was doubtful. The method of Arrighi and Hsu⁶, with modifications suggested by C. E. Ford and E. P. Evans, was used. The dry slides were incubated at 70 °C overnight, placed in warm 2× SSC (0.3 M NaCl and 0.03 M Na citrate) and returned to the incubator for 90 min. They were then stained for 3–5 min in 1 : 20 Giemsa-phosphate buffer, pH 6.8, rinsed with fresh buffer and air dried. The best results were obtained when the slides were 1–2 weeks old.

Because of the age of the animals, 2.5 times as many females were required in the second experiment as in the first in order to obtain sufficient oocytes in second metaphase. There was an average of 11 eggs per mouse. Of the oocytes collected 39% proceeded into metaphase II (Table 1). Young females had an average of 36 eggs each, with 25% proceeding into metaphase II. The increased proportion of aged cells in metaphase II is highly significant ($P < 0.0001$). On the other hand, significantly more oocytes of young mice remained in metaphase I ($P < 0.0001$). Because the oocytes were teased out of the ovaries, these differences probably reflect variations in age of the cells collected. In young mice there is probably a wider distribution of oocytes between early and late dictyotene which could affect the timing of *in vitro* division. Moreover, as a mouse ages the less hardy oocytes may have degenerated *in vivo*.

Table 1 Frequencies of oocytes collected in first metaphase and second metaphase after 18–23 h *in vitro* culture

	Irradiated					Non-irradiated				
	Total	MI	MII	Other stages	Degenerated, no response	Total	MI	MII	Other stages	Degenerated, no response
Aged mice	5,957	430(7.2)	2,269(38.1)	25(0.4)	3,233(54.3)	6,207	399(6.4)	2,518(40.6)	36(0.6)	3,254(52.4)
Young mice	8,046	789(9.8)	2,064(25.7)	34(0.4)	5,159(64.1)	7,667	889(11.6)	1,866(24.3)	24(0.3)	4,888(63.8)
Total	14,003	1,219(8.7)	4,333(30.9)	59(0.4)	8,392(60.0)	13,874	1,288(9.3)	4,384(31.6)	60(0.4)	8,142(58.7)

MI, metaphase I; MII metaphase 2. Figures in parentheses are percentages.

by exposing them to a low dose of whole body gamma irradiation from a ¹³⁷Cs source. We have now found that radiosensitivity increases with age.

Virgin female (C₃H×ICR/Swiss)F₁ hybrids, similar to those used in the previous experiment, were irradiated at age 12 months and killed within a week of exposure. The same procedure as before was followed for irradiating the females and handling controls. As before, there was no exposure to gonadotrophic hormones and the radiation dose (10–30 R) was kept low to minimise chromosome breakage. The details for obtaining second metaphase chromosome preparations have been described before⁵. Oocytes were

Comparisons between the frequencies of hyperhaploid cells in young and aged mice are shown in Table 2. Hyperhaploidy occurred in irradiated samples at a significantly higher frequency than in controls. When ageing alone is considered, frequencies of nondisjunctional events increased with age, but the increases were not significant for either irradiated or non-irradiated mice. When these numbers are doubled as an estimate of the complementary hypohaploid cells, all comparisons are highly significant. The incidence of nondisjunction among aged controls was 0.6%, increasing to 2.7% on irradiation. The nondisjunction rate for irradiated young females was 1%.

Table 2 Frequencies of numerical aberrations in second meiotic metaphase oocytes of aged mice compared with young mice, irradiated and non-irradiated

	Dose (R)	Irradiated (I)				Non-irradiated (C)			
		No. of oocytes analysable	No. of chromosomes			No. of oocytes analysable	No. of chromosomes		
			19	20	21		19	20	21
Aged mice* (12 months)	10	390	11	370	7	411	16	390	1
	20	376	12	357	5	545	14	524	0
	30	351	18	321	3	350	15	330	3
	Totals	1,117	41	1,048	15	1,306	45	1,244	4
Young mice† (3–6 months)	Totals	1,149	34	1,109	6	1,054	31	1,023	0

For comparison of hyperhaploid cells only—young I against young C, $P = 0.02$; old C against young C, $P = 0.09$ (Fisher exact test); old I against old C, $P < 0.01$; all I against all C, $P < 0.001$; all I against young I, $P < 0.07$; χ^2 with Yates' correction for small numbers.

* Cells with chromatid breaks omitted.

† Details in ref. 1.

Table 3 Frequencies of chromatid breaks and separations

	Dose (R)	Irradiated			Non-irradiated		
		No. analysable	No. with breaks	No. with separations	No. analysable	No. with breaks	No. with separations
Aged mice	10	390	2	15	411	4	27
	20	376	2	25	545	7	21
	30	351	9	12	350	2	19
Totals		1,117	13	52	1,306	13	67
Young mice	10	426	0	2	379	0	5
	20	368	0	3	421	0	2
	30	355	0	11	254	0	2
Totals		1,149	0	16	1,054	0	9

The frequency of chromatid breaks in the oocytes exposed to 30 R (Table 3) was significantly higher than the total number of breaks found in cells exposed to lower doses ($P < 0.01$) and in non-irradiated controls ($P < 0.03$). Two of these cells had translocations (Fig. 1), and one other chromatid exchange occurred in a cell exposed to 20 R. There were no differences in breakage rates between the controls and cells exposed to 10 R or 20 R. In this sample the critical dose causing a substantial increase in chromatid breaks in the oocytes of aged mice seems to be higher than 20 R. It is difficult, however, to obtain accurate breakage frequencies in metaphase II cells because fragments can be lost during first anaphase. No chromatid breaks were identified in metaphase II chromatids of young females. The frequency of premature chromatid separation was increased in aged mice whether irradiated or not (Table 3). The primary purpose of this experiment, however, was to evaluate the effect of radiation on meiotic chromosome segregation.

Yamamoto *et al.*⁷ found spontaneous and induced non-disjunction among the progeny of aged females but not of young mice. Lünig *et al.*⁸ examined the effects of low dose X-ray exposure and age of females on the rate of intrauterine death, used as an indication of aneuploid offspring. They found that late maternal age at the time of mating had a considerable effect on foetal death rate but no detectable influence was noted from X-ray exposure with doses of 2–32 R. Reichert *et al.*⁹ induced chromosome anomalies with X rays and found nondisjunctional products among second metaphase oocytes of irradiated females and none in controls: the difference, however, was not significant. They did not exclude the possibility that X rays induce abnormal segregation.

Our results with mouse oocyte chromosomes indicate that abnormal segregation may be induced during first meiotic division by *in vivo* exposure of mouse ovaries to low doses of radiation. This radiosensitivity seems to increase with age; the increase in abnormal segregation is more than twice the frequency found in young mice. This evidence in the mouse supports the suggestion that the risk of producing trisomic offspring among humans is increased with exposure of the abdomen to diagnostic X rays. Human radiation response has also been found to be related to late maternal age^{3,4}. Examination of the results of 11 epidemiological studies¹⁰ indicates that all but two^{11,12} show an increase in nondisjunction with radiation exposure, although in some series these increases were not statistically significant.

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Photo-oxidation of water in phospholipid bilayer membranes containing chlorophyll *a*

THE process of electron transfer from water to NAD in living photosynthetic systems involves a crystalline or liquid crystalline two-dimensional lattice of chlorophyll and other pigments. We describe here an artificial model system, with one of the characteristics of pigment system II, in which oxidation of water under the influence of red light (680 nm) is mediated by chlorophyll incorporated into bilayer membranes.

There have been several investigations of the photosensitivity of chlorophyll incorporated into phospholipid bilayers. For example, when chloroplast extracts were incorporated into the phospholipid bilayer separating two aqueous solutions of different redox potential, photocurrents through the bilayer were observed^{1–3}. A peak was observed at 680 nm, corresponding to the red absorption of chlorophyll *a*⁴. Nicholls *et al.* incorporated chlorophyll *a* into lipid liposomes and observed the photooxidation of added ferrocytochrome *c*⁵, and Tomkiewicz *et al.* observed photoproduction of the chlorophyll *a* cation radical in the presence of the water-soluble oxidants⁶. This suggests that redox reactions take place at the membrane-solution interface under the influence of light absorption by chlorophyll *a*. We report here light-induced oxidation of water in aqueous dispersions of phospholipid liposomes containing chlorophyll *a* and *b* together with small amounts of other pigments within the chloroplasts of spinach leaves.

To confirm that photo-induced oxygen production takes place at one surface of the bilayer membrane and is associated

with a redox reaction at the other surface, we used liposome dispersion systems containing water-soluble oxidants only in the aqueous compartment inside each liposome.

A preparation consisting of chlorophyll *a* (68.0 weight %) and *b* (22.8 weight %), and pigments such as carotene and xanthophyll (9.2 weight %) was prepared from spinach leaves by repeated precipitation in a methanol-dioxane-water mixture in the appropriate proportions⁷. Phosphatidylcholine was isolated chromatographically from egg yolk⁸. Liposome dispersions were prepared by suspending colyophilised phosphatidylcholine (100 mg) with a given amount of the chlorophyll preparation and a trace of carbonylcyanide *D*-trifluoromethoxyphenyl hydrazone (FCCP) in a 5-ml aqueous solution of appropriate composition. The suspension was irradiated ultrasonically with a sonicator at 20 kHz and 4 °C under argon for 15 min. Undispersed phospholipids were removed by centrifugation at 13,000*g* for 10 min. The resulting supernatant was subjected to molecular sieve chromatography at 4 °C on a column of Sepharose 4 B. The elution profile of the dispersion consisted of two distinct peaks. The position of the second peak was independent of the molar ratio of chlorophyll *a* to phosphatidylcholine in our experimental conditions and was similar to that observed for liposome dispersion without chlorophyll. Thus the liposomes in this peak were about 250 Å in diameter⁹. To prepare a system in which oxidants existed only in the aqueous interior of each liposome, sonication was carried out in an aqueous solution of 0.1 M potassium ferricyanide, 0.5 M Tris-chloride and 0.1 M potassium chloride at pH 7.50. $K_3Fe(CN)_6$ was removed from the external aqueous phase by rapid passage of the liposome dispersion at 4 °C over a column of Sephadex G-50 which had been equilibrated with the aqueous solution of 0.5 M Tris-chloride and 0.1 M KCl at pH 7.50. A sample of 5 ml of the resulting dispersion was transferred to a glass cell with an oxygen electrode connected to a high speed electrometer (417 High Speed Picoammeter, Keithley, Fig. 1).

Fig. 1 Schematic diagram of experimental setup for measurement of light-induced oxygen production in the chlorophyll-liposome system. E, electrometer; R, recorder; GP, gum plug; OE, oxygen electrode; KOH, 0.1 M KOH solution; PB, Pb electrode; PT, Pt electrode; TM, Teflon membrane; EN, black enamel layer; D, dispersion of liposomes; W, window; S, stirring bar; MR, rotary magnet.

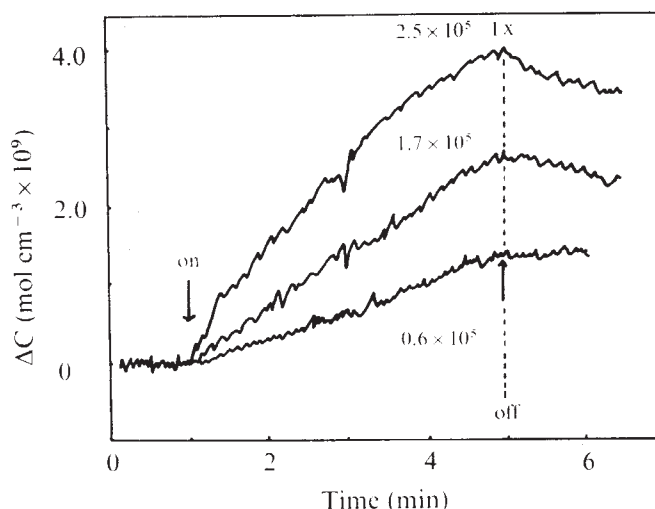
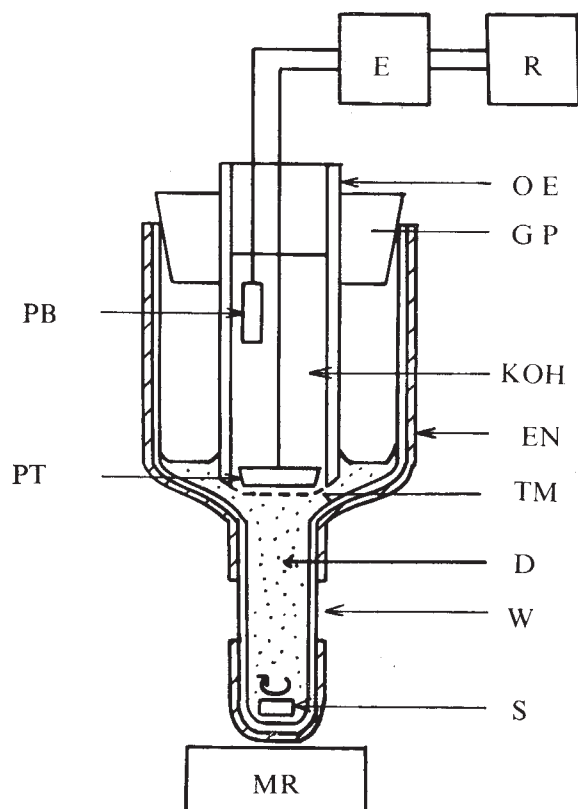
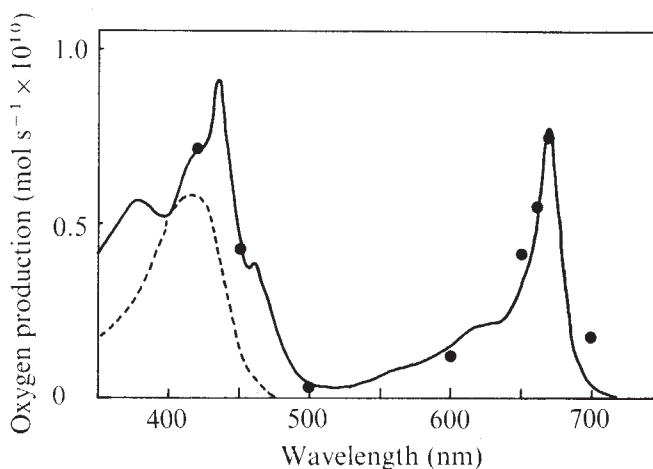


Fig. 2 Change of oxygen concentration in the liposome dispersion when illuminated by white light of three different intensities. Numbers represent light intensity in lx.

The outside of the cell was coated with a thin layer of black enamel to protect it from stray light, except for a window of 1.2 cm² through which the liposome system was irradiated with light. In particular the oxygen electrode was not exposed to light. Before irradiation with light, the dispersion was bubbled with Ar gas for 20 min to remove dissolved oxygen. A 500-W xenon lamp with an infrared cut off glass filter was used throughout the experiments and light intensity was varied by means of neutral density filters. A monochromator or a set of interference filters was used as needed.

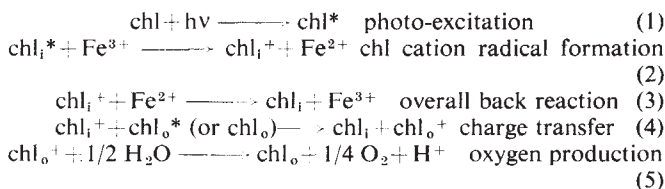
Figure 2 illustrates the change in oxygen concentration in the external aqueous phase under white light illumination at various intensities. The data were obtained in a dispersion of the liposomes composed of 100 mg of phosphatidylcholine and 1.0 mg of chlorophyll preparation. The rate of oxygen production calculated from the initial slope of each line was nearly proportional to the light intensity. The rate of oxygen production at 100,000 lx was $4.2 \times 10^{-11} \text{ mol l}^{-1}$. Figure 3 compared the action spectrum of the photoproduction of oxygen in the same system with the absorption spectra of chlorophyll incorporated in the liposome and of $K_3Fe(CN)_6$ in aqueous solution. The position of the red band of chlorophyll *a* in the liposome bilayer was similar to that observed for chlorophyll *a* in ethanol solution. As Fig. 3 shows,

Fig. 3 Comparisons of the action spectrum for oxygen production with the absorption spectra of the chlorophyll sample in the liposome bilayer and of $K_3Fe(CN)_6$ in aqueous solution. ●, Action spectrum for oxygen production; —, absorption spectrum of the chlorophyll sample in the liposome bilayer; ---, absorption spectrum of $K_3Fe(CN)_6$ in aqueous solution.



the action spectrum has two peaks around 420 nm and 670 nm, corresponding to the absorption bands of chlorophyll *a* in the liposome bilayer, but has no similarity to the absorption spectrum of $K_3Fe(CN)_6$. These results suggest that the reaction proceeds by utilising photoenergy absorbed by chlorophyll. In the absence of $K_3Fe(CN)_6$ in the compartment of each liposome, oxygen production was scarcely observed even in the light.

Tomkiewicz *et al.* observed the photoproduction of the chlorophyll cation radical in the dispersion of liposomes containing chlorophyll *a* in the presence but not in the absence of $K_3Fe(CN)_6$ (ref. 6). Taking their results into consideration ours might be expressed



In these equations, chl^* and chl^+ are excited chlorophyll *a* and chlorophyll *a* cation radical, respectively, and Fe^{3+} and Fe^{2+} represent ferricyanide and ferrocyanide ions. chl_i and chl_o are the chlorophyll *a* molecules localised in membrane surfaces facing the aqueous phase inside each liposome and the ambient aqueous phase outside the liposome, respectively.

The explanation of these equations is as follows. Chlorophyll *a* is excited by light at both membrane-solution interfaces (equation (1)), but chl^+ is produced only inside the liposomes (equation (2)). The back reaction may occur at the internal surface (equation (3)). Competing with this back reaction, charge exchange between chl_i^+ and chl_o^* (or chl_o) might take place through a redox species present in the membrane, such as carotene and xanthophyll¹⁰ (equation (4)). The chl_o^+ thus generated in the outer surface may oxidise water in the external aqueous phase; consequently oxygen is produced in the ambient aqueous phase. The excess negative charge accumulated inside each liposome by the charge exchange between chl_i^+ and chl_o^* (or chl_o) may be compensated by FCCP-mediated proton transport from the external to the internal aqueous solution of the liposomes.

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Structure of nosiheptide, a polythiazole-containing antibiotic

NOSIHEPTIDE is a metabolite with a strong antibiotic action which has been isolated from *Streptomyces actuosus* (F. Benazet *et al.*, unpublished). Studies of its antibiotic spectrum and cross-resistance suggested that it belonged to the thiostrepton family¹; chemical analyses and further chemical degradation confirmed this view. From analytical and spectroscopic data (NMR ¹H, ¹³C and ¹⁵N) the general formula $C_{51}H_{43}N_{13}O_{12}S_6$ was assigned

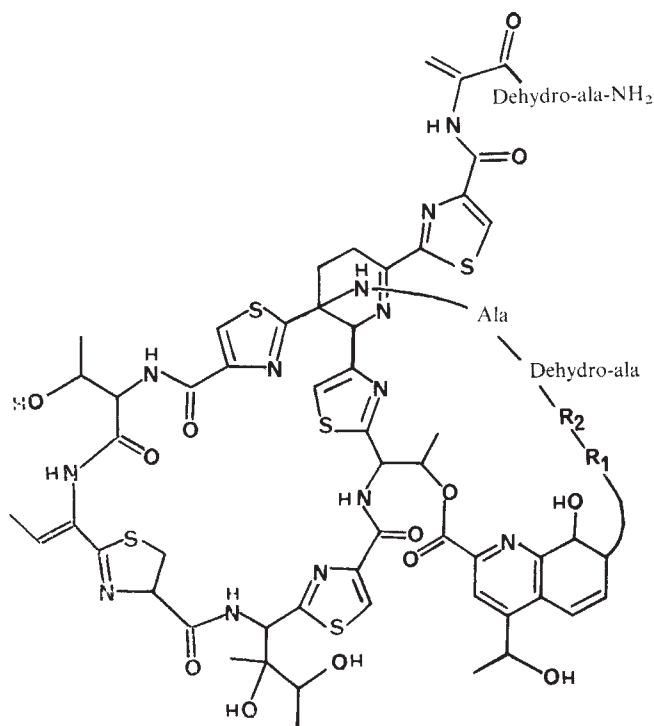
(A. Brun, H. Depaire, G. Lukacs and J. P. Thomas, unpublished). Unfortunately no M^+ peak can be extracted from its mass spectrum (electron impact). Some parts of the molecule have been identified after degradation by different techniques, namely one L-threonine, one hydroxypyridine, five thiazoles and one indole ring (A. Brun, H. Depaire, G. Lukacs, and J. P. Thomas, unpublished). Nothing could be deduced about the sixth sulphur atom. At least twenty antibiotics are known to contain thiazole rings (for instance bleomycins², bacitracin A², and althiomycin³), but among those containing five thiazole rings only two molecular structures are known: thiostrepton, by X-ray analysis⁴ and ¹³C NMR spectroscopy, and siomycin A, by comparing its NMR spectrum with that of thiostrepton⁵. To obtain the complete structure of nosiheptide, we undertook an X-ray analysis.

Crystals of nosiheptide were grown in a mixture of $CHCl_3$ and C_2H_5OH (3:1) by slow evaporation at 30 °C. Any attempt to change the ratio of the two solvents led to powdery precipitates or unusable small crystals. They were mounted in Lindemann glass capillaries in the presence of solvent which is part of the overall crystal structure, as these crystals decompose as soon as the mother liquor is removed. Crystal data: $C_{51}H_{43}N_{13}O_{12}S_6$, molecular weight = 1,222, tetragonal, space group $I4_1$; $a = b = 36.05$, $c = 11.44$ Å, $Z = 8$ and $V = 14,735$ Å³. (It is noteworthy that thiostrepton, with a similar molecular structure, was first found in the tetragonal system before it was crystallised in the monoclinic form.) A rapid decrease in intensities was found at higher diffraction angles and only 2,740 reflections (spacing limit θ up to 60°) could be collected by a computer-controlled diffractometer using $CuK\alpha$ radiation (filtered by a graphite monochromator) in the $\theta/2\theta$ scanning mode. Intensities were corrected for the effects of slow decomposition and by the Lorentz and polarization factors but not for absorption.

The measured density is 1.41 g cm⁻³, as in thiostrepton. The calculated density, with eight molecules in the cell, is 1.107 g cm⁻³. Consequently there is a difference of 336 in total mass of the asymmetric unit which is attributable to the solvent.

Attempts to find the trial structure by the sharpened Patterson method were unsuccessful. The structure was solved with some difficulty by the combined use of the phase function⁶

Fig. 1 Condensed formula for thiostrepton (R_1 – R_2 = Ile–Ala) and siomycin A (R_1 – R_2 = Val–Dehydro-ala). (From ref. 5.)



(to select the starting set) and the multiresolution method⁷ (for tangent refinement). Starting with the four highest peaks of the best E map, regarded as sulphur atoms, a long carbon chain was revealed. A second cycle led to the location of the pyridine ring and the three bonded thiazole rings. Further recycling revealed the complete structure. Identification of the different atoms was made on the basis of chemical evidence (A. Brun, H. Depaire, G. Lukacs and J. P. Thomas, unpublished), temperature factors, bond distances and angles. Details of the crystallographic problems encountered will be published later.

Full matrix least-squares refinement with isotropic temperature factors gave a conventional residual *R* of 25%. The sulphur atoms alone were then assumed to be anisotropically vibrating. Because of the small number of reflections, the rest of the molecule was kept isotropic. The final *R* was 18% with a Cruickshank⁸ weighting scheme.

No significant peaks could be recognised in the last difference Fourier synthesis (maximum peak $1 \text{ e } \text{\AA}^{-3}$) except weak peaks attributed to one CHCl_3 molecule with a low occupation factor. The rest of the solvent was therefore assumed to be randomly distributed.

The formula of nosiheptide is shown in Fig. 2 and may be compared with that of thiostrepton (Fig. 1). Some interesting differences are seen: (1) The tetrahydro pyridine cycle (anchorage part of the molecular chains) as well as the thiazoline ring of the thiostrepton backbone, are unsaturated in nosiheptide. Consequently the three chains can be linked to each other in a more planar conformation than the four chains of thiostrepton. Following this observation, the upper part of the molecule is conjugated to the pyridine ring, allowing it to be nearly coplanar, and therefore is much less agitated than the proposed side chain of thiostrepton. The presence of a free hydroxyl group on the pyridine ring might be used to prepare chemical derivatives for further biochemical studies. (2) The quinaldic precursor (quinoline derivative) is replaced by a methyl-indolic compound of probable similar biosynthetic origin, but differently bridged over the chains. (3) The sixth sulphur atom is found in an unusual thiol-ester linkage. This was rather

Fig. 2 Nosiheptide, general formula. Chains are attached together by three asymmetric centres which all have the S configuration, deduced from the L-threonine residue.

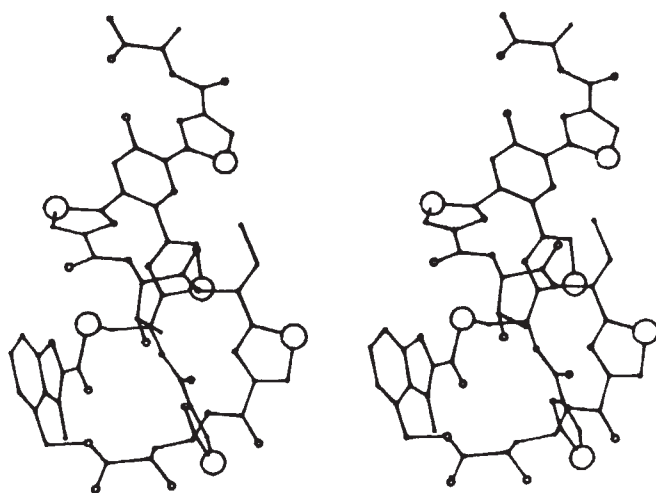
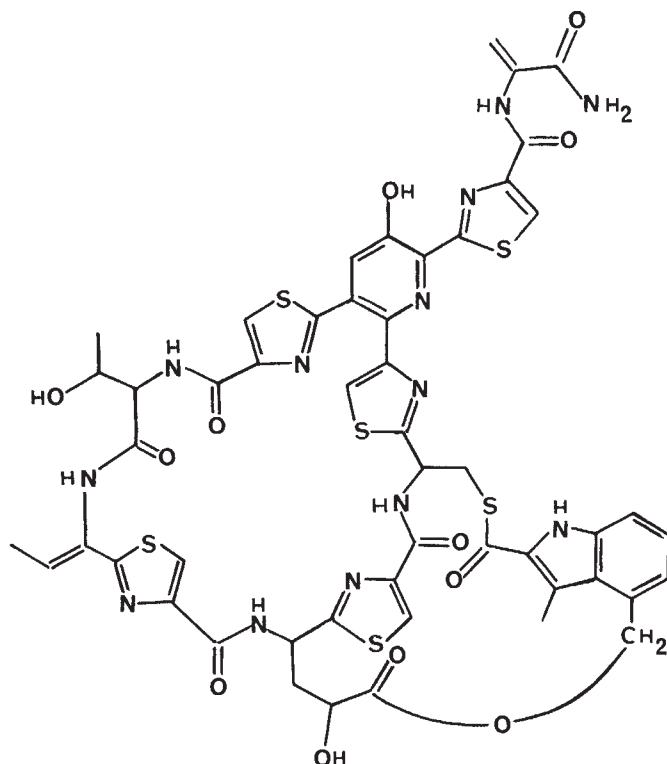


Fig. 3 Stereoscopic view of nosiheptide. The general globular conformation of thiostrepton is very similar, despite the absence of the fourth chain. Drawings of the molecular packing of this structure reveal large void channels in which solvent is in complete disorder.

unexpected on the basis of initial thiostrepton analogies and is, to our knowledge, the first thiol-ester function isolated in an antibiotic compound.

A stereoscopic view of the molecule is given in Fig. 3 and clearly shows the linkage of the indolic part over the macrocycle constituted by thiazole rings.

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Erratum

In the article 'Specific binding sites for oestrogen at the outer surfaces of isolated endometrial cells' by Richard J. Pietras and Clara M. Szego (*Nature*, **265**, 71; 1977), the ordinate labels to Fig. 3 should read from 0 to 80, not from 10 to 90.

reviews

Institutionalised science in America

J. B. Morrell

The Pursuit of Knowledge in the Early American Republic: American Scientific and Learned Societies from Colonial Times to the Civil War. Edited by A. Oleson and S. C. Brown. Pp. xxv+372. (Johns Hopkins: Baltimore and London, July 1976.) £11.55.

IN 1769 the American Philosophical Society (Philadelphia), the oldest learned society in the USA, was reorganised into its present form under the presidency of Franklin, who had originally founded it in 1743. Less than a century later, America had produced two national scientific societies: the voluntary American Association for the Advancement of Science (AAAS, founded 1848), and the federally sponsored National Academy of Sciences (founded 1863). After the Civil War both local and national scientific societies were eventually replaced by universities, federal bureaux, specialist societies, and private foundations, as the principal means of discovering, certifying, and diffusing scientific knowledge. Yet in the first hundred years of the American republic, the learned society, devoted to general science, *belles lettres*, technical instruction, agricultural improvement, or the promotion of manufactures, constituted the chief genre of organisation through which knowledge was pursued.

The eighteen papers in this volume were commissioned by the American Academy of Arts and Sciences as the first phase of a collaborative study of the history of institutionalised science and learning in America. By focusing on the scientific centres of the eastern states, such as Philadelphia, Boston, New York and Albany, several contributors confirm the importance of regional differences. The historical significance of local context is, however, best revealed by Shapiro's lively analysis of the Western Academy of Sciences at Cincinnati, the Porkopolis of the mid-West. The theme of scientific knowledge as public utility, conceived patriotically and democratically, is also well covered. Indeed almost all the contributions, which are adequately indexed, deal competently with the staple features of institutional history;

and they bristle with dedicated documentation.

By the end of the nineteenth century American science had become 'professional' in several ways: arcane specialisation was rampant; training procedures in laboratories and lecture rooms were well established; qualifications of scientific competence were publicly available and recognised; and many full-time paid jobs in science were filled by certificated individuals. Quite naturally several contributors have been enticed by the theme of professionalisation, the obvious example being Kohlstedt in her account of the ways in which the AAAS reflected and shaped the allegedly professional pattern of the 1850s.

Other authors, however, will have none of this. In a forthright essay Reingold stresses the social and intellectual inclusiveness of most *ante bellum* scientific societies, and accordingly he offers a speculative taxonomy of the scientific community divided into researchers, practitioners, and cultivators. Shapiro's essay erodes the professionalisation model even further by rejecting as unpardonably anachronistic any attempt to divide the membership of American scientific societies into distinct occupations before about 1850.

Irrespective of their attitude to whether professionalisation is a valid organising theme, most contributors present overwhelming evidence to show that many societies failed to fulfil their own stated purposes and that some declined into paralysed, helpless, and hopeless organisations. Especially in a democratic country, where the privilege of learned societies was sometimes suspect, their establishment, maintenance, and growth required the persistent attention of devoted individuals who saved them from precarious existence or quiet extinction. When the continuing existence of so many societies was a pervasive problem, one feels that the professionalisation model is an inappropriate key to their history. Rather one suspects that the origins, purposes, and features of these societies may be interpreted in terms of the various interests, ideas, and resources of the participating and sometimes conflicting members. At least, one would expect that scientific societies faced

difficulty in translating aims into action.

For these reasons the most interesting essays are those which deal with failure or which give considerable weight to the aims and perceptions of individual scientists. Hindle, for instance, accounts for the failure of New Yorkers to establish a viable scientific society rivalling those of Philadelphia and Boston by arguing that they preferred to expend their energies in various societies for practical arts modelled on the London Society of Art and not the Royal Society.

Gerstner's analysis of the changing features of the Philadelphia Academy of Natural Sciences reveals the dominance from 1817 to 1840 of William Maclure and the subsequent challenge to it. Sinclair gives similar coherence to his study of the Franklin Institute of Philadelphia by showing how its multifarious activities of the 1820s were redirected by Alexander Dallas Bache into experimental technical research, publication, and public funding. Starting from the assumption that scientific societies only exist when they serve the needs of members as well as those of science, Shapiro shows that in the Cincinnati Academy the unresolved conflicts about classification led to its evanescence in 1854.

Although Kohlstedt employs the professionalisation model, her results reveal the persistent feuding within the early AAAS between the advocates of social inclusiveness led by Henry Darwin Rogers and the elite group of Lazzaroni committed to exclusiveness. Not surprisingly the latter led by Bache became dis-satisfied with the AAAS, and found the highly select National Academy of Sciences far more congenial.

All these contributions confirm that during the past ten years or so American historians have consolidated the history of American science as a lively field, distinguished generally by competence and occasionally by *éclat*. This volume is an admirable testament to that enviable achievement. □

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Problems of quantum biochemistry

Quantum Biochemistry and Specific Interactions. By Z. Simon. Pp. 251. (Abacus: Tunbridge Wells, Kent, UK, 1976.) £11.95.

APPLYING the methods of quantum chemistry to problems in biochemistry is so fraught with difficulties that high levels of scepticism are required in addition to enthusiasm for the subject. Until very recently theoretical chemists were not notably successful in calculating properties of small isolated molecules let alone large molecules in aqueous solution or in even more complicated environments.

Over the past few years the problems of molecular physics have become tractable due to refinements of molecular orbital techniques and the power of computers. With the confidence gained from success with the isolated molecule, theoreticians are becoming more interested in biological problems; in particular conformational studies and comparisons of reactivity are proving valuable. Professor Simon's book, originally published in Romanian in 1973, was written before the advances of the past few years, but must have been almost out of date even then.

The molecular orbital technique described in the chapter which follows the introduction is almost totally devoted to π -electron Hückel theory; a final section on 'advanced methods' lists variants more likely to be referred to as 'crude' in current publications; and neither *ab initio* nor PCIO approaches are mentioned. None of the many references cited is later than 1973 despite the fact that the work is described as a 'revised, up-dated translation'. It is an elaboration of the work of the Pullmans in their 1963 book on quantum biochemistry rather than the total revision which would be much welcomed.

Similarly, the chapter on intermolecular forces rests heavily on work by the Pullmans and Scheraga, which both groups would probably consider to have been superseded. Reactivities are discussed in terms of parameters deduced from π -electron calculations rather than the potential surfaces currently in vogue.

The specific interactions referred to in the title and an analysis of some recognition processes occupy the last two chapters of the book. Enzyme reactions, repressor-operator interactions and specificity in immune phenomena are all considered, but much more space is devoted to descriptions of the

topics and to quantitative measures than to applications of quantum mechanical methods.

The strength of the book is its collection of facts and topics of biochemistry which remain as problems rather than a description of how they have been solved. The descriptive portion like the theory suffers from the time lag in appearance of the work, but it is impossible to read without feeling an urge to tackle some of the problems with more up-to-date methods.

The book is in no way an account of the state of the art but it is a valuable collection of topics and references more to be recommended to the seasoned campaigner than to the student.

Graham Richards

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Whirlpool of aggression

The Evolution and Chemistry of Aggression. By D. D. Thiessen. Pp. xviii+211. (Charles C. Thomas: Springfield, Illinois, 1976.)

THE first chapter of this book is entitled "The Evolution of Competitive Behavior". The other four are on gene organisation, endocrine control, chemical social signals, and the actions of drugs. There is a bibliography of about 300 entries, and a very brief subject index. The author's stated intention is to investigate aggression in relation to evolution and chemistry. "Unless otherwise noted", he writes, "aggression will refer to intermale aggression."

I read the first chapter in some bewilderment. "Aggression," we are told, "in one form or another, is as old as life itself." The first pages include passages on 'molecular aggression', competition between species, predator-prey interactions, and species diversification. There follows a section on "a move toward intraspecific competition" devoted largely to the unusual conduct of a species of sea anemone. There is then one on brain differentiation, in which there are references to 'competitive readjustment of brain size' and 'selection for neurological competence to deal with distance perception'.

Dr Thiessen evidently equates aggression with competition. "If an animal fails to fight", he tells us, "we too readily conclude that he is non-aggressive. . . . Even an animal that does not fight . . . may still be highly

competitive." In some passages competition seems to be thought of as equivalent to what goes on in the private sector. The biological principles that deal with competition are those of population genetics, but they are disregarded. Instead, we have tautologies: for example, "there is positive selection for those forms that optimize their use of space and commodities and negative selection for those forms that fail." Similarly, none of the recent critical reviews on natural selection in relation to behaviour, or on intolerant behaviour within species, are represented in the bibliography.

Dr Thiessen's chapter on his own speciality, the endocrine control of behaviour, summarises much interesting work, but again without a unifying theme. Among the many topics mentioned are sex reversal in fish and mammals, the disorganisation of sexual development by early treatment with hormones, the metabolism of the hypothalamus, hormones and social status, and human chromosome abnormalities. There is also a discussion of the behavioural changes that accompany the human menstrual cycle.

Dr Thiessen is not always uncritical. In this field the danger of inferring causal relationships from correlations is always with us. For example, violent prisoners have high plasma testosterone levels. Dr Thiessen remarks that the violence may not be due to the hormone: instead imprisonment could increase testosterone secretion. And on page 82 he utters an important warning: "specialised definitions of aggression involving violence and sexual assault may be more amenable to investigation than more generalised notions of aggression."

This principle could have been usefully applied to the chapter on drugs. "A survey of the literature", we learn, "indicates that almost all classes of drugs studied have been found to decrease, increase or not influence aggression." This is not surprising when we find, a few pages later, a list which includes "footshock aggression" (probably defensive postures induced by pain), "isolation aggression" (possibly a response to strangeness), "irritable aggression" due to brain injury, "aggression" induced by various drugs, and predatory behaviour.

The editor of the American Lecture Series, Dr I. N. Kugelmass, writes in his foreword: "What is aggression? It is life itself—a whirlpool in many ways. When once set agoing it spins on and on." As Australians say, too right.

S. A. Barnett

S. A. Barnett is Professor of Zoology at the Australian National University, Canberra.

obituary

Luigi Gorini, who was a professor emeritus of microbiology and molecular genetics at Harvard Medical School died on August 13 at the age of 72 in Boston. Luigi was born in Milan, in 1903. Although he received his scientific training in the 1920s, he did not really enter his research career until after the second World War. Because he was one of the few scientists who refused to pledge an oath to the fascist government of Italy in the 1930s, he was unable to secure academic employment. He eventually joined the anti-fascist underground.

At the end of the war in Italy, Luigi was a prime mover in establishing a refuge for the many children who were orphaned. Restricted by lack of money and facilities, Luigi decided that the most needy children were Jewish orphans and he set up and ran the home at Selvino for them prior to their emigration to Israel. In 1976, the account of these activities by Luigi and his wife Annamaria Torriani was laid in the Martyrs and Heroes Archives at Yad-Vashem, Israel.

His first scientific paper was published in 1946 when he was 42 years old. Much of Luigi's early work was on the mechanism of protection of various bacterial proteases by ions such as calcium and manganese. He and his co-workers were able to show that the metal ions protected these enzymes against autodigestion by stabilising particular protein conformations. This work had wide impact in that it provided a strong suggestion that proteins do not have unique folding patterns, but can exist in several different stable states.

Upon coming to the United States, Luigi's interests shifted to problems of gene regulation in *Escherichia coli*. In collaboration with Werner Maas at New York University, he used the chemostat to study steady state growth of several arginine auxotrophs with and without arginine. In this way they discovered a large derepression effect on the arginine pathway and were able to show elegantly how repression and feedback inhibition could interact to exert a coarse and fine tuning effect on the rate of enzyme synthesis and arginine formation in that pathway. These studies helped to establish the concept of end-product repression and derepression in biosynthetic pathways. Luigi and his coworkers later continued to contribute to the understanding of

regulation of arginine biosynthesis.

After moving to Harvard in 1957, Luigi's research interests expanded to a study of the effect of streptomycin on the translation of messenger RNA into protein. By demonstrating that streptomycin and mutations of the ribosome to streptomycin resistance could influence the accuracy of translation, Gorini and Kataja provided, in 1964, the first evidence that ribosomes were not merely passive templates for protein synthesis. Rather, ribosomes played an important role in determining the interaction between transfer RNA and messenger RNA. These proposals were confirmed directly by *in vitro* experiments in which it was shown that ribosomes sensitive to streptomycin promote mistakes in reading of the code in the presence of streptomycin and other aminoglycoside antibiotics. Subsequently, in 1969, Rosset and Gorini showed that it was possible to increase misreading by the ribosomes through altering a ribosomal protein by the *ram* (ribosomal ambiguity) mutation.

Thus again this large body of work brought Gorini's focus directly on to a basic mechanism for the control of all growth—this time at the level of translation of the gene code. His subsequent work centred around two of the components at this step—the ribosome and transfer RNA. Many of his results in this area were presented in a review on Informational Suppression in 1970 (*A. Rev. Genet.*, **4**, 107–134). His last projects were concerned with establishing an active role for streptomycin with the ribosome or on one of its components, and on the possible interaction of ribosomes in the coupled transcription-translating system.

In 1949, Luigi was awarded the Kronauer Prize by the Faculty of Science at the Sorbonne, and he won the Ledlie Prize of Harvard University in 1965. He was elected a member of the National Academy of Sciences in 1971.

Luigi remained active in his laboratory until the last few months before his death, both advising people in the laboratory and doing experiments himself. One remarkable feature of his scientific life is that, between the time of his official retirement age of 65 at Harvard Medical School and his death, he published 33 papers.

Luigi also remained active politically. He had many letters published in the

newspapers protesting policies he opposed. When Henry Kissinger was awarded the Nobel Prize for Peace in 1973, Luigi organised a petition protesting this award. The petition was sent to the Nobel Committee and received publicity in the United States. He also was very active in the anti-Vietnam War movement and spoke out constantly against the use of genetic arguments to support racial discrimination. The following excerpt from a speech he gave in 1970 at Montana State University gives an idea of his conception of the political role of scientists:

"My job here tonight is to make you realise that for me, like for hundreds of us scientists, my own scientific interest means a lot intellectually but, morally speaking, science alone does not satisfy entirely my conscience. I will try to be the most unequivocal radical possible and at the same time constructive, so that when I quit, your opinion about me should not be similar to that expressed a long time ago by the fascist Italian police about someone whom I know after his first confrontation with them. He was very happy to be released, for a time at least, but a few years later he discovered by chance the written motivation for letting him out and he was really not satisfied. The police file sounds like the following: 'Lonely anarchist; he is not dangerous.'"

The following are excerpts from a conversation between the two of us, J. B. who worked in the same department as Luigi, and M. D. who worked in his laboratory.

M.D. Politics probably shared equal prominence in his life with science.

J.B. He would be horrified when people would do things that didn't fit in with his sense of morality. He never lost his sense of outrage.

M.D. Things were black or white to him. In a way it was a great strength of character. He would never compromise.

J.B. Very frequently in the morning he would come rushing down the hall into my office, holding a newspaper or asking me if I'd seen or heard the news that morning. We had to do something right away about it—compose a letter . . .

M.D. . . . or send off a telegram.

J.B. Or sometimes when he got home he had heard something on the news and he was so upset that he would have to call people. I connect his continued sense of outrage about anything political that upset him with his intense enthusiasm and involvement in his scientific work. He never mellowed in that sense.

M.D. He was always in the lab. before anyone else. He was so enthusiastic that he would have got everyone's plates out of the incubator before they came in.

J.B. It seems amazing to me that he maintained that involvement until the end—that it never dissipated.

M.D. He was talking once about a scientist who was retiring and was going to retire to live in Europe. He said "How can a man live like that without science? If I didn't have something to look forward to every day, what would my life be?"

He was not encouraging in that he would say: "That was a really nice experiment". But it was his enthusiasm that was so overwhelming. He tried to have a personal relationship with everyone in the lab. And discussions about projects, although sometimes heated, were usually fruitful, amicable, encouraging and often hilarious.

J.B. He was physically very active. A few years ago he was climbing mountains in the Dominican Republic.

M.D. We went to Mount Washington once, we drove to the top. We climbed down a way and then climbed back up to the car. He was really amazing. He was walking at a good pace, while everyone else was struggling up.

J.B. He was always telling jokes or funny stories. When he entered this country as an immigrant, he had to answer some questions. One of the questions was about adultery. Luigi answered the interviewer by asking "Do you want a yes or no answer or number of times?"

M.D. He was such a good teacher to people in the lab. about life and about everything.

**Jon Beckwith
Margaret Duncan**

John Donald Rose, FRS, who died on October 14 was a former Research and Development Director of Imperial Chemical Industries Ltd. He was born on January 2, 1911 in Rotherham, near Sheffield and went to Rotherham Grammar School before reading chemistry at Jesus College, Oxford. Throughout his life, he never lost a certain bluff "no nonsense" Yorkshire

earthiness. He was also a man of quite exceptional kindness and generosity of spirit, and had a well-developed sense of the ludicrous. The combination of these qualities was exceedingly attractive and it was well-nigh impossible not to like "J.D."

He took a First in chemistry at Oxford, and was awarded a Salters' Institute Fellowship, part of which he chose to spend working in Prof. L. Ruzicka's laboratory at the Eidgenössische Technische Hochschule in Zurich. He joined the Research Department of the Dyestuffs Division of ICI at Blackley in 1935 and is believed to have been the first person to make a sample of Nylon 66 in this country, just as he was later to be one of the first ICI men to develop Whinfield's recently discovered polyethylene terephthalate ("Terylene"). During the war he was a member of, and later head of an Exploratory Research Section, and carried out a thorough investigation of the chemistry of the nitroparaffins. At the end of the war, he was deeply involved in the detailed examination of unpublished German war-time research, and his good working knowledge of German made him a valuable interrogator. Characteristically he was kind and generous to German scientists who were being held as possible war criminals. Thereafter, he was rapidly promoted, becoming Research Director of the Dyestuffs Division in 1951.

It was during his term as Research Director that Rattee and Stevens discovered the reactive cotton dyes, the "Procions." J.D. knew very well that the ICI patent position and technological lead were not initially very strong, and he showed great courage in deploying a major effort, fully supported by his colleagues on the Development side, to get the "Procions" on to the market very quickly—in fact within two years. Considerable faith was needed to persuade the dyeing industry to adopt totally new techniques and concepts, but the venture succeeded and the first three dyestuffs were soon expanded into a full range.

In 1959 he was transferred to the Paints Division as joint Managing Director, becoming Chairman in 1964. In 1966 he joined the ICI Board as member responsible for research and development. He was elected Fellow of the Royal Society in 1971 in recognition of "his contributions to chemical science and its utilisation in industry." After his retirement in 1972 he held office as Master of the Salters' Company (1973), an honour which gave him particular pleasure.

It would be wrong to suggest that J.D. was an outstandingly original scientist—he never claimed to be one.

His talent was a great clearness of mind, sound judgement and the ability to guide and encourage those associated with him. Many ICI scientists would gladly agree that their own developments or discoveries owe something, often far from negligible, to him.

He died at the age of 65, leaving a widow, son and daughter, and four grandchildren.

M. A. T. Rogers

Dr Alfred E. Emerson, the world famous termitologist died on Sunday October 3, 1976 at Glenn Falls, New York.

He was born in Ithaca and was educated there, at Cornell University. In 1922 he became an Associate Professor in the Department of Zoology at Pittsburgh University, but moved to Chicago in 1929, to remain there for the rest of his academic career, becoming an Emeritus Professor in 1962.

His major research was on the taxonomy of termites, regarded by the uninitiated merely as pests, but to Dr Emerson fascinating social creatures, possessing, as they do, a large measure of self-sacrifice, a trade union system that runs with a minimum of demarcation disputes, and very sophisticated engineering—some termites air-condition their nests by humidity and heat control (sometimes by using the metabolic heat production of fungi deliberately brought into the nest for the purpose) and by the cunning design of interconnecting air shafts.

In his research he travelled extensively, living on occasion in tribal villages in the remote jungles of Borneo and the Congo, capturing, cataloguing and classifying termites. His collection, built up over half a century of expeditions, comprises of 1,645 species (to Emerson's chagrin, only 93 per cent. of the known world total) and is preserved, pickled in alcohol, in the Museum of Natural History in New York, to whom Emerson presented it in 1964. It has already proved invaluable to termitologists from all over the world. The fascination of the termite world is conveyed by a book *Termite City* which, together with his wife, née Eleanor Fish, he wrote for children.

This was just one of his numerous publications, dealing with his primary research, evolution (he was a proponent of the controversial but now largely discredited theory of the Superorganism) and ecology. He became interested in ecology long before it was fashionable; he was elected president of the Ecological Society of America in 1941, and, amongst the many honours showered on him, was named 'Eminent Ecologist' by them in 1967.